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FILE 'USPAT' ENTERED AT 11:54:17 ON 09 AUG 1998

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  *              W E L C O M E   T O   T H E                        *
  *         U. S.   P A T E N T   T E X T   F I L E                 *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

=> s (antibod?)(P)(7b6 or 16c10 or 7c10 or 20c9)
```

```
          29671 ANTIBOD?
             12 7B6
              1 16C10
              8 7C10
              3 20C9
L1            3 (ANTIBOD?)(P)(7B6 OR 16C10 OR 7C10 OR 20C9)

=> d l1 1-3


1.  5,723,309, Mar. 3, 1998, Production of subunits of soluble T cell
receptors by co-transfection; Marc Bonneville, 435/69.1, 4, 7.1, 69.52,
69.7, 172.3 [IMAGE AVAILABLE]

2.  5,702,910, Dec. 30, 1997, Method of sandwich immunoassay for
N-peptide; Yoshito Numata, et al., 435/7.94, 7.8, 7.9, 7.92, 7.95, 176,
960; 436/518, 542, 547, 548, 811 [IMAGE AVAILABLE]

3.  5,179,000, Jan. 12, 1993, Method for assaying basic fetoprotein in
urine and assay kit therefor; Masaru Ishii, et al., 435/7.23, 7.1, 7.2,
7.9, 7.92; 436/64, 518, 813 [IMAGE AVAILABLE]

=> d l1 1-3 kwic


US PAT NO:      5,723,309 [IMAGE AVAILABLE]             L1: 1 of 3

DRAWING DESC:

DRWD(8)

 FIG. 4 represents the titration in soluble TR activity expressed in
.mu.g/ml, as attested by the IRMA test (sandwich 7B6/TiV.delta.2), of
the fractions eluted from an affinity column coupled with the
anti-V.gamma. 7B6 antibody (marketed by Immunotech), onto which
have been applied about 500 ml of supernatant from the culture of
.gamma..delta.sFS-CHO cells.

DRAWING DESC:

DRWD(9)

 FIG. 5 represents the SDS-PAGE analysis of fractions positive for the
soluble TR activity, as attested by the IRMA test (sandwich
7B6/TiV.delta.2), of the fractions eluted from an affinity column
coupled with the anti-V.gamma.9B6 antibody.

DETDESC:

DETD(16)

 Immulon-1 microtitre plates (Dynatech, Marnes, France) were coated for
90 min at 37.degree. C. with 50 .mu.l of Y102 (or 7B6) monoclonal
antibody at 40 .mu.g/ml in a phosphate buffered saline solution.
After removal of the antibody, the unbound sites were saturated with
a phosphate buffered saline solution containing 0.5% bovine serum albumin
for 1 hour at.  .  .  were then added in an amount of 40 .mu.l at the
same time as 10 .mu.l of labelled TiV.delta.2 monoclonal antibody.
After incubating for 90 min at 37.degree. C., the wells were rinsed four
times with 100 .mu.l of a phosphate.  .  .

DETDESC:

DETD(17)
```

The bound radioactivity was measured in a .gamma. scintillation counter. The following set of **antibodies** was used to measure the secretion of soluble TR.gamma..delta. by the IRMA technique: anti-V.gamma.9 (Y102, B37, **7B6**), anti-C.gamma. (B121) and anti-V.delta.2 (TiV.delta.2) **antibodies** (Miossec et al., 1989, J. Exp. Med. 171:1171). A monoclonal **antibody** specific for IL2 was also used as negative control.

DETDESC:

DETD(20)

10 mg of Y102 or **7B6** monoclonal **antibody** (anti-V.gamma.9) were covalently linked to a matrix of activated agarose beads (Affigel, Biorad, Richmond, Calif.) according to the instructions of. . .

DETDESC:

DETD(90)

The purification described previously for isolating the V.gamma.9V.delta.2 receptor consisted of an immunopurification with an anti-V.gamma.9 **antibody** (Y102 or **7B6**). An affinity column of the same type but using the **antibody** 510 described above and which recognizes a determinant of the delta constant chain was used. The advantage of this new. . .

US PAT NO: 5,702,910 [IMAGE AVAILABLE] L1: 2 of 3

SUMMARY:

BSUM(12)

In another embodiment of the present invention, the first or second monoclonal **antibody** is **7B6**.

SUMMARY:

BSUM(13)

In another embodiment of the present invention, one of the first and second monoclonal **antibodies** is **7B6**, and the other is a monoclonal **antibody** recognizing a portion of the amino acid sequence of the N-peptide, of which the recognition site is different from that of the **7B6**.

SUMMARY:

BSUM(17)

In another embodiment of the present invention, the first monoclonal **antibody** is KY-ANP-III, and the second is **7B6**.

SUMMARY:

BSUM(21)

In one embodiment of the present invention, the monoclonal **antibody** is **7B6**.

DRAWING DESC:

DRWD(3)

FIG. 1B shows reactivity of monoclonal **antibody 7B6** of the

present invention with N-peptide(1-25) and N-peptide(43-67).

DETDESC:

DETD(39)

B. Production of a Hybridoma Producing **7B6 Antibody** and Production of the **7B6 Antibody** Using the Hybridoma

DETDESC:

DETD(42)

A sub-class of **antibodies** produced by the hybridomas was determined by using the culture supernatant of the hybridomas. A mouse monoclonal **antibody** isotyping kit (Amersham Corp.) was used for this determination. A monoclonal **antibody** newly obtained was designated as **7B6**, belonging to the isotype IgG.sub.1 (.kappa.).

DETDESC:

DETD(43)

The reactivities of the known monoclonal **antibody** KY-ANP-III (IgG.sub.1 .kappa.) and the new monoclonal **antibody 7B6** with various kinds of N-peptide fragments were confirmed by the above-mentioned EIA method. As the KY-ANP-III, a monoclonal **antibody** produced by a hybridoma FERM BP-1887 was used. The results are shown in FIG. 1. In this figure, .largecircle. represents reactivity with N-peptide(1-25) and .circle-solid. represents reactivity with N-peptide(43-67). The KY-ANP-III **antibody** reacted with N-peptide(1-25), but not with N-peptide(43-67) (FIG. 1A). In contrast, the **7B6 antibody** reacted with N-peptide(43-67), but not with N-peptide(1-25) (FIG. 1B). Thus, it was confirmed that two **antibodies** recognize different portions of N-peptide, respectively, that is, the KY-ANP-III **antibody** recognizes the 1-25 amino acid sequence of N-peptide and the **7B6 antibody** recognizes the 43-66 portion of N-peptide.

DETDESC:

DETD(44)

The hybridoma producing the monoclonal **antibody 7B6** of the present invention was deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of. . .

DETDESC:

DETD(47)

Example 2: Purification of Monoclonal **Antibody 7B6** from Ascites of a Mouse

DETDESC:

DETD(48)

Two . . . minutes. The precipitate thus obtained was dissolved in PBS and dialyzed against PBS at 4.degree. C. overnight to give purified **7B6** monoclonal **antibodies**.

DETDESC:

DETD(51)

Example 4: Labelling of **7B6 Antibody** with Radioactive Iodine (.sup.125 I)

DETDESC:

DETD(52)

A purified **7B6 antibody** was labelled with .sup.125 I by the chloramine T method as follows:

DETDESC:

DETD(53)

First, . . . 2 mCi/20 .mu.l of Na.sup.125 I (Amersham Corp.) were placed in a glass tube. Then, 60 .mu.l of 3.3 mg/ml **7B6 antibody** solution in PBS and 20 .mu.l of 1 mg/ml chloramine T solution in 0.5M phosphate buffer (pH 7.5) were added. . . was applied on Superose 12 (1.times.30 cm) (Pharmacia) and eluted with 0.1M phosphate buffer (pH 7.0) to give .sup.125 I-labelled **7B6 antibody** fractions.

DETDESC:

DETD(55)

Standard . . . Kathon CG (Rohm and Haas) was added to each test tubes. Each one of the beads coated by the KY-ANP-III **antibody** produced in Example 3 was added to the test tube. Each test tube was shaken at 200 strokes per minute. . . 0.1% Kathon CG). Then, a second reaction was conducted as follows. Three hundred .mu.l (about 200,000 cpm) of .sup.125 I-labelled **7B6 antibody** solution diluted with Buffer D containing 0.1% BSA was added to each test tube, and the test tubes were shaken. . .

DETDESC:

DETD(65)

Example 6: Labelling of **7B6 Antibody** with Horseradish Peroxidase (HRP)

DETDESC:

DETD(66)

A purified **7B6 antibody** was labelled with HRP by a method of Ishikawa et al., supra, as follows:

DETDESC:

DETD(67)

First, . . . 2.2 mg/ml pepsin (Boehringer-Mannheim) solution in 0.2M citrate buffer (pH 4.0) were added to 4.5 ml of 4.4 mg/ml purified **7B6 antibody** solution in PBS, and the mixture was incubated at 37.degree. C. for 18 hours. The resulting digest in the mixture. . .

DETDESC:

DETD(72)

Fifty . . . Then, 250 .mu.l of Buffer C was added to each test tube. Each one of beads coated by the KY-ANP-III **antibody** produced in Example 3 was added to each test tube. Each test tube was horizontally

shaken at 200 strokes per. . . times with 2 ml of Buffer D. Then, the second reaction was conducted as follows. Three hundred .mu.l of HRP-labelled **7B6 antibody** solution diluted with Buffer D containing 0.1% BSA was added to each test tube (200 ng/test tube), and the test. . .

CLAIMS:

CLMS(3)

3. A method according to claim 1 or 2, wherein the first or second monoclonal **antibody** is **7B6**.

CLAIMS:

CLMS(4)

4. A method according to claim 3, wherein one of the first and second monoclonal **antibodies** is **7B6**, and the other is a monoclonal **antibody** that binds a portion of the amino acid sequence of the .gamma.-hANP(1-98), of which a binding site is different from that of the **7B6**.

CLAIMS:

CLMS(8)

8. A method according to claim 7, wherein the first monoclonal **antibody** is KY-ANP-III, and the second monoclonal **antibody** is **7B6**.

US PAT NO: 5,179,000 [IMAGE AVAILABLE] L1: 3 of 3

SUMMARY:

BSUM(21)

antigenic determinant	monoclonal **antibody**
A	5C4, 5C5, 5C6, 7D1, K1
B	5B3, 5C2
C	5A2, 5A3, 7D3, 8A2, 7B4, 5D6-2, 8A1, 7A5, 8A5, **7B6**

=> s (b7 or b7(w)1)(P)(antibod?)(P)(ctla(w)4)

```
          4993 B7
          4993 B7
       2323337 1
         29671 ANTIBOD?
            60 CTLA
       2266864 4
L2            6 (B7 OR B7(W)1)(P)(ANTIBOD?)(P)(CTLA(W)4)
```

=> d l2 1-6

1. 5,770,704, Jun. 23, 1998, Receptor activation with inactive hepatocyte growth factor ligands; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

2. 5,763,584, Jun. 9, 1998, Receptor activation with hepatocyte growth

factor agonists; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

3. 5,756,096, May 26, 1998, Recombinant antibodies for human therapy; Roland A. Newman, et al., 424/154.1, 133.1, 141.1; 530/387.1 [IMAGE AVAILABLE]

4. 5,747,034, May 5, 1998, Methods and materials for the induction of T cell anergy; Mark de Boer, et al., 424/137.1, 141.1, 156.1; 435/70.21, 172.2, 329, 343.1, 343.2; 530/387.1, 387.3, 387.5, 388.1, 388.85, 809 [IMAGE AVAILABLE]

5. 5,684,136, Nov. 4, 1997, Chimeric hepatocyte growth factor (HGF) ligand variants; Paul J. Godowski, 530/399, 387.3 [IMAGE AVAILABLE]

6. 5,576,423, Nov. 19, 1996, Antibodies to the slam protein expressed on activated T cells; Gregorio Aversa, et al., 530/388.75; 424/154.1; 435/70.21, 172.3, 331, 343.2; 530/387.9, 389.6, 391.3 [IMAGE AVAILABLE]

=> d l2 1-6 date

L2: 1 of 6

TITLE: Receptor activation with inactive hepatocyte growth factor ligands
US PAT NO: 5,770,704 DATE ISSUED: Jun. 23, 1998 [IMAGE AVAILABLE]
APPL-NO: 08/792,078 DATE FILED: Jan. 31, 1997
REL-US-DATA: Continuation of Ser. No. 423,291, Apr. 17, 1995, abandoned, which is a division of Ser. No. 268,880, Jun. 30, 1994, abandoned, which is a continuation of Ser. No. 950,572, Sep. 22, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L2: 2 of 6

TITLE: Receptor activation with hepatocyte growth factor agonists
US PAT NO: 5,763,584 DATE ISSUED: Jun. 9, 1998 [IMAGE AVAILABLE]
APPL-NO: 08/435,764 DATE FILED: May 5, 1995
REL-US-DATA: Continuation of Ser. No. 87,784, Jul. 13, 1993, abandoned, which is a continuation-in-part of Ser. No. 950,572, Sep. 21, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and a continuation-in-part of Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L2:d l2 1-6 kwic

3 of 6

TITLE: Recombinant antibodies for human therapy
US PAT NO: 5,756,096 DATE ISSUED: May 26, 1998 [IMAGE AVAILABLE]
APPL-NO: 08/476,237 DATE FILED: Jun. 7, 1995
REL-US-DATA: Continuation-in-part of Ser. No. 379,072, Jan. 25, 1995, Pat. No. 5,658,570, which is a continuation of Ser. No. 912,292, Jul. 10, 1992, abandoned, which is a continuation-in-part of Ser. No. 856,281, Mar. 23, 1992, abandoned, which is a continuation-in-part of Ser. No. 735,064, Jul. 25, 1991, abandoned.

L2: 4 of 6

TITLE: Methods and materials for the induction of T cell anergy
US PAT NO: 5,747,034 DATE ISSUED: May 5, 1998 [IMAGE AVAILABLE]

APPL-NO: 08/200,716 DATE FILED: Feb. 18, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 15,147, Feb. 9, 1993, which is a continuation-in-part of Ser. No. 910,222, Jul. 9, 1992, Pat. No. 5,397,703.

L2: 5 of 6

TITLE: Chimeric hepatocyte growth factor (HGF) ligand variants
US PAT NO: 5,684,136 DATE ISSUED: Nov. 4, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/435,501 DATE FILED: May 5, 1995
REL-US-DATA: Continuation of Ser. No. 87,784, Jul. 13, 1993, abandoned, which is a continuation-in-part of Ser. No. 950,572, Sep. 21, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L2: 6 of 6

TITLE: Antibodies to the slam protein expressed on activated T cells
US PAT NO: 5,576,423 DATE ISSUED: Nov. 19, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/348,792 DATE FILED: Dec. 2, 1994

=>

US PAT NO: 5,770,704 [IMAGE AVAILABLE] L2: 1 of 6

DETDESC:

DETD(74)

Chimeras . . . 2221-2229 (1990); Watson et al., Nature 349, 164-167 (1991)]; CD44* [Aruffo et al., Cell 61, 1303-1313 (1990)]; CD28 * and **B7*** [Linsley et al., J. Exp. Med. 173, 721-730 (1991)]; **CTLA-4*** [Lisley et al., J. Exp. Med. 174, 561-569 (1991)]; CD22* [Stamenkovic et al., Cell 66. 1133-1144 (1991)]; TNF receptor [Ashkenazi. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,763,584 [IMAGE AVAILABLE] L2: 2 of 6

DRAWING DESC:

DRWD(91)

Chimeras . . . 110, 2221-2229 (1990); Watson et al., Nature 3, 164-167 (1991)1; CD44* (Aruffo et al., Cell 61, 1303-1313 (1990)); CD28* and **B7*** (Linsley et al., J. Exp. Med. 173, 721-730 (1991)); **CTLA-4*** (Lisley et al., J. Exp. Med. 174, 561-569 (1991)); CD22* (Stamenkovic et at., Cell 66. 1133-1144 (1991)); TNF receptor (Ashkenazi. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,756,096 [IMAGE AVAILABLE] L2: 3 of 6

DETDESC:

DETD(26)

In using the subject CE9.1 monoclonal **antibody** for the treatment of autoimmune disorders, including for example rheumatoid arthritis, this **antibody** may be administered by itself or in combination with other compounds suitable for treatment of the particular disease condition. For example, the subject **antibody** may be administered in combination with

other proteins, for example monoclonal **antibody** soluble receptor proteins to TNF-alpha, monoclonal **antibodies** to IL2 receptor, monoclonal **antibodies** and receptor fusion proteins which antagonize the CD40/gp39 interaction and **CTLA 4**-Ig in monoclonal **antibodies** which antagonize the **B7**/CD28 interaction. Also, in the case of treatment of rheumatoid arthritis, the subject **antibody** may be administered in combination with other therapeutics, for example Rapamycin, Leflunomide, Tenidap, RS-61443 (Mycophenolate Mofetil), Surenyl (sodium Hyaluronate), anti-TCR (V.beta.17) peptide vaccine, Anerva X (anti-MHC vaccine), and extracorporeal protein A immunoabsorbants or combinations thereof. Additionally, the subject **antibody** may be administered in combination with other **antibodies** produced according to the invention or known in the art which are specific to human CD4. This may result in synergistic effects, for example, if these **antibodies** bind to different epitopes of the CD4 protein.

US PAT NO: 5,747,034 [IMAGE AVAILABLE] L2: 4 of 6

DETDESC:

DETD(4)

Monoclonal **antibody** B7-24 is an unique monoclonal **antibody** that binds specifically to the B7-1 molecule, but not to B7-2. This is in contrast with a recombinant fusion protein of the **CTLA-4** molecule (Linsley, J. Exp. Med., 174, 561 (1991), which binds to both B7-1 and B7-2. Monoclonal **antibody** B7-24 is also different from the anti-**B7** monoclonal **antibody** BB-1, which binds to B7-1 and in addition to a third form of the **B7** molecule, B7-3 (Boussiotis et al., Proc. Nat'l. Acad. Sci. (USA), 90, 11059 (1993)). Although it is known that both B7-1. . . T cells by binding to the CD28 molecule, it is not known that blocking only B7-1 with a specific monoclonal **antibody** such as B7-24, when combined with an immunosuppressive drug such as cyclosporin A, can induce T-cell tolerance or anergy. This. . .

US PAT NO: 5,684,136 [IMAGE AVAILABLE] L2: 5 of 6

DETDESC:

DETD(74)

Chimeras . . . 110, 2221-2229 (1990); Watson et al., Nature 349, 164-167 (1991)); CD44* (Aruffo et al., Cell 61, 1303-1313 (1990)); CD28* and **B7*** (Linsley et al., J. Exp. Med. 173, 721-730 (1991)); **CTLA-4*** (Lisley et al., J. Expo. Med. 174, 561-569 (1991)); CD22* (Stamenkovic et al., Cell 66. 1133-1144 (1991)); TNF receptor (Ashkenazi. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,576,423 [IMAGE AVAILABLE] L2: 6 of 6

SUMMARY:

BSUM(4)

The . . . N.Y. Increased adhesion between T cells and antigen presenting cells (APC) or other forms of primary stimuli, e.g., immobilized monoclonal **antibodies** (mAb), can potentiate the T-cell receptor signals. T-cell activation and T cell expansion depends upon engagement of the T-cell receptor. . . Today 11:211-216; and Jenkins (1994) Immunity 1:443-446. A major, and well-studied, co-stimulatory interaction for T cells involves either CD28 or **CTLA-4** on T cells with either **B7** or B70 (Jenkins (1994) Immunity 1:443-446). Recent studies on CD28 deficient mice (Shahinian, et al. (1993) Science

261:609-612; Green, et al. (1994) Immunity 1:501-508) and **CTLA-4** immunoglobulin expressing transgenic mice (Ronchese, et al. (1994) J. Exp. Med. 179:809-817) have revealed deficiencies in some T-cell responses though. . .

=> d his

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     (FILE 'USPAT' ENTERED AT 11:54:17 ON 09 AUG 1998)
L1             3 S (ANTIBOD?)(P)(7B6 OR 16C10 OR 7C10 OR 20C9)
L2             6 S (B7 OR B7(W)1)(P)(ANTIBOD?)(P)(CTLA(W)4)

=> s (b7 or b7(w)1)(P)(antibod?)(P)(cd28)

          4993 B7
          4993 B7
       2323337 1
         29671 ANTIBOD?
           147 CD28
L3          24 (B7 OR B7(W)1)(P)(ANTIBOD?)(P)(CD28)

=> s l3(P)(human?)

        183156 HUMAN?
L4          14 L3(P)(HUMAN?)

=> d l3 1-24
```

1. 5,770,704, Jun. 23, 1998, Receptor activation with inactive hepatocyte growth factor ligands; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

2. 5,770,197, Jun. 23, 1998, Methods for regulating the immune response using B7 binding molecules and IL4-binding molecules; Peter S. Linsley, et al., 424/134.1, 139.1, 144.1, 192.1, 810; 435/69.7; 530/350, 388.7, 868 [IMAGE AVAILABLE]

3. 5,763,584, Jun. 9, 1998, Receptor activation with hepatocyte growth factor agonists; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

4. 5,756,096, May 26, 1998, Recombinant antibodies for human therapy; Roland A. Newman, et al., 424/154.1, 133.1, 141.1; 530/387.1 [IMAGE AVAILABLE]

5. 5,747,037, May 5, 1998, Anti-GP39 antibodies; Randolph J. Noelle, et al., 424/154.1, 130.1, 141.1, 143.1, 144.1, 153.1, 173.1; 435/70.21, 172.2, 326, 332, 334, 343, 343.1, 343.2, 346; 530/387.1, 388.1, 388.2, 388.22, 388.7, 388.73, 388.75 [IMAGE AVAILABLE]

6. 5,747,034, May 5, 1998, Methods and materials for the induction of T cell anergy; Mark de Boer, et al., 424/137.1, 141.1, 156.1; 435/70.21, 172.2, 329, 343.1, 343.2; 530/387.1, 387.3, 387.5, 388.1, 388.85, 809 [IMAGE AVAILABLE]

7. 5,741,899, Apr. 21, 1998, Chimeric receptors comprising janus kinase for regulating cellular pro liferation; Daniel J. Capon, et al., 536/23.4; 435/69.7, 320.1, 325, 377; 530/350, 387.3 [IMAGE AVAILABLE]

8. 5,723,127, Mar. 3, 1998, Compositions and methods for use of IL-12 as an adjuvant; Phillip Scott, et al., 424/184.1, 191.1, 204.1, 234.1, 269.1; 530/350 [IMAGE AVAILABLE]

9. 5,718,883, Feb. 17, 1998, Transgenic animal model for autoimmune

diseases; David M. Harlan, et al., 424/9.2; 435/172.3; 514/2; 800/2, DIG.1 [IMAGE AVAILABLE]

10. 5,712,149, Jan. 27, 1998, Chimeric receptor molecules for delivery of co-stimulatory signals; Margo R. Roberts, 435/252.3, 69.7, 320.1; 530/350; 536/23.4 [IMAGE AVAILABLE]

11. 5,707,624, Jan. 13, 1998, Treatment of Kaposi's sarcoma by inhibition of scatter factor; Brian J. Nickoloff, et al., 424/158.1, 143.1, 145.1, 152.1 [IMAGE AVAILABLE]

12. 5,691,135, Nov. 25, 1997, Immunoglobulin superantigen binding to gp 120 from HIV; Jonathan Braun, et al., 435/5, 7.1, 7.2, 7.24, 7.92, 974 [IMAGE AVAILABLE]

13. 5,686,281, Nov. 11, 1997, Chimeric receptor molecules for delivery of co-stimulatory signals; Margo R. Roberts, 435/172.3, 7.1, 7.2, 69.7; 536/23.4 [IMAGE AVAILABLE]

14. 5,684,136, Nov. 4, 1997, Chimeric hepatocyte growth factor (HGF) ligand variants; Paul J. Godowski, 530/399, 387.3 [IMAGE AVAILABLE]

15. 5,652,224, Jul. 29, 1997, Methods and compositions for gene therapy for the treatment of defects in lipoprotein metabolism; James M. Wilson, et al., 514/44; 424/93.21; 435/172.3, 320.1, 325, 354, 366, 369, 370 [IMAGE AVAILABLE]

16. 5,637,481, Jun. 10, 1997, Expression vectors encoding bispecific fusion proteins and methods of producing biologically active bispecific fusion proteins in a mammalian cell; Jeffrey A. Ledbetter, et al., 435/69.6, 69.1, 69.7, 172.1, 320.1, 326, 328, 332 [IMAGE AVAILABLE]

17. 5,633,234, May 27, 1997, Lysosomal targeting of immunogens; J. Thomas August, et al., 514/44; 424/185.1, 192.1; 435/69.3, 252.3, 320.1; 530/350, 395, 806; 536/23.4, 23.5 [IMAGE AVAILABLE]

18. 5,580,756, Dec. 3, 1996, B7IG fusion protein; Peter S. Linsley, et al., 435/69.7, 91.1; 530/350, 387.1, 387.3, 395; 536/23.4 [IMAGE AVAILABLE]

19. 5,576,423, Nov. 19, 1996, Antibodies to the slam protein expressed on activated T cells; Gregorio Aversa, et al., 530/388.75; 424/154.1; 435/70.21, 172.3, 331, 343.2; 530/387.9, 389.6, 391.3 [IMAGE AVAILABLE]

20. 5,571,515, Nov. 5, 1996, Compositions and methods for use of IL-12 as an adjuvant; Phillip Scott, et al., 424/208.1, 204.1, 234.1; 530/350 [IMAGE AVAILABLE]

21. 5,525,503, Jun. 11, 1996, Signal transduction via CD28; Christopher E. Rudd, et al., 435/375; 530/330 [IMAGE AVAILABLE]

22. 5,521,288, May 28, 1996, CD28IG fusion protein; Peter S. Linsley, et al., 530/387.3; 435/7.2, 7.92, 69.1, 69.7, 91.1, 252.3, 252.33, 320.1; 530/300, 350, 387.1, 395, 409, 866, 867, 868; 536/23.1, 23.4, 23.53 [IMAGE AVAILABLE]

23. 5,474,897, Dec. 12, 1995, Screening assay for the identification ov novel immunosuppressives using cultured T cells; Arthur Weiss, et al., 435/6, 69.1, 70.4 [IMAGE AVAILABLE]

24. 5,434,131, Jul. 18, 1995, Chimeric CTLA4 receptor and methods for its use; Peter S. Linsley, et al., 514/2; 424/133.1; 514/12; 530/350, 866, 868; 935/10 [IMAGE AVAILABLE]

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L3: 1 of 24

TITLE: Receptor activation with inactive hepatocyte growth factor ligands
US PAT NO: 5,770,704 DATE ISSUED: Jun. 23, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/792,078 DATE FILED: Jan. 31, 1997
REL-US-DATA: Continuation of Ser. No. 423,291, Apr. 17, 1995, abandoned, which is a division of Ser. No. 268,880, Jun. 30, 1994, abandoned, which is a continuation of Ser. No. 950,572, Sep. 22, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L3: 2 of 24

TITLE: Methods for regulating the immune response using B7 binding molecules and IL4-binding molecules
US PAT NO: 5,770,197 DATE ISSUED: Jun. 23, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/008,898 DATE FILED: Jan. 22, 1993
REL-US-DATA: Continuation-in-part of Ser. No. 723,617, Jul. 27, 1991, abandoned.

L3: 3 of 24

TITLE: Receptor activation with hepatocyte growth factor agonists
US PAT NO: 5,763,584 DATE ISSUED: Jun. 9, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/435,764 DATE FILED: May 5, 1995
REL-US-DATA: Continuation of Ser. No. 87,784, Jul. 13, 1993, abandoned, which is a continuation-in-part of Ser. No. 950,572, Sep. 21, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and a continuation-in-part of Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L3: 4 of 24

TITLE: Recombinant antibodies for human therapy
US PAT NO: 5,756,096 DATE ISSUED: May 26, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/476,237 DATE FILED: Jun. 7, 1995
REL-US-DATA: Continuation-in-part of Ser. No. 379,072, Jan. 25, 1995, Pat. No. 5,658,570, which is a continuation of Ser. No. 912,292, Jul. 10, 1992, abandoned, which is a continuation-in-part of Ser. No. 856,281, Mar. 23, 1992, abandoned, which is a continuation-in-part of Ser. No. 735,064, Jul. 25, 1991, abandoned.

L3: 5 of 24

TITLE: Anti-GP39 antibodies
US PAT NO: 5,747,037 DATE ISSUED: May 5, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/475,847 DATE FILED: Jun. 7, 1995
REL-US-DATA: Continuation-in-part of Ser. No. 232,929, Apr. 25, 1994, abandoned, which is a continuation-in-part of Ser. No. 116,255, Sep. 2, 1993, abandoned.

L3: 6 of 24

TITLE: Methods and materials for the induction of T cell anergy
US PAT NO: 5,747,034 DATE ISSUED: May 5, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/200,716 DATE FILED: Feb. 18, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 15,147, Feb. 9, 1993, which is a continuation-in-part of Ser. No. 910,222,

Jul. 9, 1992, Pat. No. 5,397,703.

L3: 7 of 24

TITLE: Chimeric receptors comprising janus kinase for regulating cellular pro liferation
US PAT NO: 5,741,899 DATE ISSUED: Apr. 21, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/481,003 DATE FILED: Jun. 7, 1995
REL-US-DATA: Continuation of Ser. No. 382,846, Feb. 2, 1995.

L3: 8 of 24

TITLE: Compositions and methods for use of IL-12 as an adjuvant
US PAT NO: 5,723,127 DATE ISSUED: Mar. 3, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/621,493 DATE FILED: Mar. 25, 1996
REL-US-DATA: Division of Ser. No. 265,087, Jun. 17, 1994, Pat. No. 5,571,515, which is a continuation-in-part of Ser. No. 229,282, Apr. 18, 1994, abandoned.

L3: 9 of 24

TITLE: Transgenic animal model for autoimmune diseases
US PAT NO: 5,718,883 DATE ISSUED: Feb. 17, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/197,790 DATE FILED: Feb. 17, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 48,042, Apr. 14, 1993, abandoned.

L3: 10 of 24

TITLE: Chimeric receptor molecules for delivery of co-stimulatory signals
US PAT NO: 5,712,149 DATE ISSUED: Jan. 27, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/383,749 DATE FILED: Feb. 3, 1995

L3: 11 of 24

TITLE: Treatment of Kaposi's sarcoma by inhibition of scatter factor
US PAT NO: 5,707,624 DATE ISSUED: Jan. 13, 1998
[IMAGE AVAILABLE]
APPL-NO: 08/253,728 DATE FILED: Jun. 3, 1994

L3: 12 of 24

TITLE: Immunoglobulin superantigen binding to gp 120 from HIV
US PAT NO: 5,691,135 DATE ISSUED: Nov. 25, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/306,116 DATE FILED: Sep. 14, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 259,669, Jun. 14, 1994, abandoned, which is a continuation of Ser. No. 9,705, Jan. 26, 1993, abandoned.

L3: 13 of 24

TITLE: Chimeric receptor molecules for delivery of co-stimulatory signals
US PAT NO: 5,686,281 DATE ISSUED: Nov. 11, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/455,860 DATE FILED: May 31, 1995
REL-US-DATA: Continuation of Ser. No. 383,749, Feb. 3, 1995.

L3: 14 of 24

TITLE: Chimeric hepatocyte growth factor (HGF) ligand variants
US PAT NO: 5,684,136 DATE ISSUED: Nov. 4, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/435,501 DATE FILED: May 5, 1995
REL-US-DATA: Continuation of Ser. No. 87,784, Jul. 13, 1993, abandoned, which is a continuation-in-part of Ser. No. 950,572,

Sep. 21, 1992, abandoned, which is a continuation-in-part of Ser. No. 884,811, May 18, 1992, Pat. No. 5,316,921, and Ser. No. 885,971, May 18, 1992, Pat. No. 5,328,837.

L3: 15 of 24

TITLE: Methods and compositions for gene therapy for the treatment of defects in lipoprotein metabolism
US PAT NO: 5,652,224 DATE ISSUED: Jul. 29, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/393,734 DATE FILED: Feb. 24, 1995

L3: 16 of 24

TITLE: Expression vectors encoding bispecific fusion proteins and methods of producing biologically active bispecific fusion proteins in a mammalian cell
US PAT NO: 5,637,481 DATE ISSUED: Jun. 10, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/121,054 DATE FILED: Sep. 13, 1993
REL-US-DATA: Continuation-in-part of Ser. No. 13,420, Feb. 1, 1993, abandoned.

L3: 17 of 24

TITLE: Lysosomal targeting of immunogens
US PAT NO: 5,633,234 DATE ISSUED: May 27, 1997
[IMAGE AVAILABLE]
APPL-NO: 08/006,845 DATE FILED: Jan. 22, 1993

L3: 18 of 24

TITLE: B7IG fusion protein
US PAT NO: 5,580,756 DATE ISSUED: Dec. 3, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/219,518 DATE FILED: Mar. 29, 1994
REL-US-DATA: Division of Ser. No. 722,101, Jun. 27, 1991, which is a continuation-in-part of Ser. No. 547,980, Jul. 2, 1990, abandoned, which is a continuation-in-part of Ser. No. 498,949, Mar. 26, 1990, abandoned.

L3: 19 of 24

TITLE: Antibodies to the slam protein expressed on activated T cells
US PAT NO: 5,576,423 DATE ISSUED: Nov. 19, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/348,792 DATE FILED: Dec. 2, 1994

L3: 20 of 24

TITLE: Compositions and methods for use of IL-12 as an adjuvant
US PAT NO: 5,571,515 DATE ISSUED: Nov. 5, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/265,087 DATE FILED: Jun. 17, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 229,282, Apr. 18, 1994, abandoned.

L3: 21 of 24

TITLE: Signal transduction via CD28
US PAT NO: 5,525,503 DATE ISSUED: Jun. 11, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/128,971 DATE FILED: Sep. 28, 1993

L3: 22 of 24

TITLE: CD28IG fusion protein
US PAT NO: 5,521,288 DATE ISSUED: May 28, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/219,116 DATE FILED: Mar. 29, 1994
REL-US-DATA: Division of Ser. No. 722,101, Jun. 27, 1991, abandoned,

which is a continuation-in-part of Ser. No. 547,980, Jul. 2, 1990, abandoned, which is a continuation-in-part of Ser. No. 498,949, Mar. 26, 1990, abandoned.

L3: 23 of 24

TITLE: Screening assay for the identification ov novel immunosuppressives using cultured T cells
US PAT NO: 5,474,897 DATE ISSUED: Dec. 12, 1995
[IMAGE AVAILABLE]
APPL-NO: 08/152,955 DATE FILED: Nov. 15, 1993
REL-US-DATA: Continuation of Ser. No. 898,639, Jun. 15, 1992, abandoned.

L3: 24 of 24

TITLE: Chimeric CTLA4 receptor and methods for its use
US PAT NO: 5,434,131 DATE ISSUED: Jul. 18, 1995
[IMAGE AVAILABLE]
APPL-NO: 08/067,684 DATE FILED: May 26, 1993
REL-US-DATA: Division of Ser. No. 723,617, Jun. 27, 1991, abandoned.

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US PAT NO: 5,770,704 [IMAGE AVAILABLE] L3: 1 of 24

DETDESC:

DETD(74)

Chimeras . . . Cell. Biol. 110, 2221-2229 (1990); Watson et al., Nature 349, 164-167 (1991)]; CD44* [Aruffo et al., Cell 61, 1303-1313 (1990)]; **CD28** * and **B7*** [Linsley et al., J. Exp. Med. 173, 721-730 (1991)]; CTLA-4* [Lisley et al., J. Exp. Med. 174, 561-569 (1991)]; CD22*. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,770,197 [IMAGE AVAILABLE] L3: 2 of 24

SUMMARY:

BSUM(20)

Expression . . . soluble derivatives of cell-surface glycoproteins in the immunoglobulin gene superfamily has been achieved for CD4, the receptor for HIV-1, and **CD28** and **B7** receptors, using hybrid fusion molecules consisting of DNA sequences encoding amino acids corresponding to portions of the extracellular domain of CD4 receptor fused to **antibody** domains (immunoglobulin .gamma.1 (Capon et al., Nature 337:525-531 (1989) (CD4) and Linsley et al., J. Exp. Med., supra (**CD28** and **B7**)).

DETDESC:

DETD(54)

The . . . is expected that CTLA4Ig will act to inhibit T cells in a manner similar to the effects observed for the anti-**CD28** **antibody**, under similar conditions in vivo. Under conditions where T cell/B cell interactions are occurring as a result of contact between T cells and B cells, binding of introduced CTLA4Ig to react with **B7** antigen positive cells, for example B cells, may interfere, i.e. inhibit, the T cell/B cell interactions resulting in regulation of. . .

DETDESC:

DETD(65)

Anti-**B7** monoclonal **antibodies** prepared as described above may be used to bind to **B7** antigen to inhibit interactions of **CD28**-positive or CTLA4-positive T cells with **B7** positive cells. Anti-CTLA4 monoclonal **antibodies** may be used to bind to CTLA4 receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

DETDESC:

DETD(118)

mAbs. Murine monoclonal **antibodies** (mAbs) 9.3 (anti-**CD28**) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-**B7** antigen) and rat mAb 187.1 (anti-mouse K chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388. . .

US PAT NO: 5,763,584 [IMAGE AVAILABLE] L3: 3 of 24

DRAWING DESC:

DRWD(91)

Chimeras . . . Cell. Biol. 110, 2221-2229 (1990); Watson et al., Nature 3, 164-167 (1991)1; CD44* (Aruffo et al., Cell 61, 1303-1313 (1990)); **CD28*** and **B7*** (Linsley et al., J. Exp. Med. 173, 721-730 (1991)); CTLA-4* (Lisley et al., J. Exp. Med. 174, 561-569 (1991)); CD22*. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,756,096 [IMAGE AVAILABLE] L3: 4 of 24

DETDESC:

DETD(26)

In using the subject CE9.1 monoclonal **antibody** for the treatment of autoimmune disorders, including for example rheumatoid arthritis, this **antibody** may be administered by itself or in combination with other compounds suitable for treatment of the particular disease condition. For example, the subject **antibody** may be administered in combination with other proteins, for example monoclonal **antibody** soluble receptor proteins to TNF-alpha, monoclonal **antibodies** to IL2 receptor, monoclonal **antibodies** and receptor fusion proteins which antagonize the CD40/gp39 interaction and CTLA 4-Ig in monoclonal **antibodies** which antagonize the **B7/CD28** interaction. Also, in the case of treatment of rheumatoid arthritis, the subject **antibody** may be administered in combination with other therapeutics, for example Rapamycin, Leflunomide, Tenidap, RS-61443 (Mycophenolate Mofetil), Surenyl (sodium Hyaluronate), anti-TCR (V.beta.17) peptide vaccine, Anerva X (anti-MHC vaccine), and extracorporeal protein A immunoabsorbants or combinations thereof. Additionally, the subject **antibody** may be administered in combination with other **antibodies** produced according to the invention or known in the art which are specific to human CD4. This may result in synergistic effects, for example, if these **antibodies** bind to different epitopes of the CD4 protein.

US PAT NO: 5,747,037 [IMAGE AVAILABLE] L3: 5 of 24

DETDESC:

DETD(97)

Studies have been performed looking at the role of **CD28**-**B7**/BB1 interactions in the induction of CD8+CTL responses. It was found that **CD28**-**B7**/BB1 interactions were necessary and sufficient for the generation of class I MHC-specific CTL (Harding, F. A. and Allison, J. P.. . . (1993) J. Exp. Med. 177:1791). It has been suggested that the ligand for CD40 may be an important inducer for **B7** (Ranheim, E. A. and Kipps, T. J. (1993) J. Exp. Med. 177:925) as shown by studies involving the inhibition of **B7** expression on normal and leukemic B cells by **antibodies** to CD40. Together, these studies indicate that anti-gp39 may block interaction of CD4+ T cells with B cells thus failing to induce the expression of **B7** that allows a B cell to efficiently activate a T cell to proliferate and produce cytokines. Taken together, this data. . . cells cognate interaction between gp39 and its ligand CD40. Signaling of CD40 on the B cells then allows upregulation of **B7**/BB1. Reciprocal interaction of **B7**/BB1 with its ligand **CD28** on the T cells then allows enhanced T cell proliferation and cytokine production. If, however, only one signal is provided. . .

US PAT NO: 5,747,034 [IMAGE AVAILABLE] L3: 6 of 24

SUMMARY:

BSUM(6)

The . . . the CD2 molecule on T cells binds to LFA-3 on APCs, but it has also been shown that binding of **antibodies** to CD2 can augment the signals provided by the TCR/CD3 complex. Other ligand pairs involved in T cell activation are LFA-1/ICAM-1, CD4/MHC-class II antigen, VLA-4/VCAM, and, most importantly, **CD28**/**B7**.

SUMMARY:

BSUM(7)

A . . . determines whether TCR-stimulation leads to full T cell activation or to T cell anergy is that generated by interaction of **CD28** on the T cells with **B7** on APCs. It is reported that in vitro cross-linking of the **CD28** molecule may rescue T cells from becoming anergic. Harding et al., Nature, 356, 607 (1992). **CD28** is a homodimeric transmembrane glycoprotein with an apparent molecular mass of 44 kDa and is a member of the immunoglobulin superfamily [Aruffo et al., Proc. Nat'l. Acad. Sci. (USA), 84, 8573 (1987)]. The **CD28** molecule is expressed on approximately 95% of CD4-positive T cells and 50% of CD8-positive T cells. Costimulation of T cells with monoclonal **antibody** to the TCR/CD3 complex and **CD28** results in greatly enhanced T cell activation. Thompson et al., Proc. Nat'l. Acad. Sci. (USA), 86, 1333-1337 (1989); June et. . . resulting in a greatly enhanced production of these lymphokines. June et al., supra; and Lindsten et al., supra. Furthermore, a **CD28**-responsive element has been demonstrated in the enhancer of the IL-2 gene, suggesting that there is also regulation at the transcriptional. . . 313 (1991) and Verwey et al., J. Biol. Chem., 266, 14179-14182 (1991). Certain models of T cell activation mediated by **CD28** are reported to be relatively resistant to inhibition with CsA. June, et al., Mol. Cell. Biol., 7, 4472-4481 (1987)

SUMMARY:

BSUM(10)

Certain molecules are reported to interfere with the interaction between the **B7** and **CD28** antigens. The soluble CTLA4-Ig fusion protein is reported to partially block this interaction. Linsley et al., J. Exp.

Med., 74, 561 (1991). Anti-**CD28 antibodies** are also reported to block this interaction. Furthermore, anti-**B7 antibodies** are known. Yokochi et al., J. Immunol., 128, 823 (1982); Freedman et al., J. Immunol., 139, 3260 (1987); Valle et. . .

DETDESC:

DETD(4)

Monoclonal **antibody** B7-24 is an unique monoclonal **antibody** that binds specifically to the B7-1 molecule, but not to B7-2. This is in contrast with a recombinant fusion protein of the CTLA-4 molecule (Linsley, J. Exp. Med., 174, 561 (1991), which binds to both B7-1 and B7-2. Monoclonal **antibody** B7-24 is also different from the anti-**B7** monoclonal **antibody** BB-1, which binds to B7-1 and in addition to a third form of the **B7** molecule, B7-3 (Boussiotis et al., Proc. Nat'l. Acad. Sci. (USA), 90, 11059 (1993)). Although it is known that both B7-1 and B7-2 can co-stimulate T cells by binding to the **CD28** molecule, it is not known that blocking only B7-1 with a specific monoclonal **antibody** such as B7-24, when combined with an immunosuppressive drug such as cyclosporin A, can induce T-cell tolerance or anergy. This. . .

DETDESC:

DETD(16)

As . . . B7-1 antigen. The complex is formed in a manner that blocks the normal signal transduction pathway of B7-1 through the **CD28** or CTLA4 antigen. Molecules which bind to the **B7** antigen include **CD28**, CTLA4, CTLA4Ig and anti-**B7 antibodies**.

DETDESC:

DETD(72)

A . . . the B7-1 antigen such as MAb B7-24; and (2) an immunosuppressive agent. Molecules that bind to the B7-1 antigen include **CD28**, CTLA4, CTLA4Ig and anti-**B7 antibodies** as described in Section III above.

DETDESC:

DETD(155)

FIG. 7 shows the inhibition of anti-CD3-induced, **B7**-mediated proliferation of T cells by anti-B7-1 Mabs B7-24 (squares) or BB-1 (triangles). FIG. 8 shows the effect of blocking **B7/CD28** interaction during secondary MLC (open symbols). Purified T cells were stimulated with the EBV-transformed B cell line ARC for 3. . . presence or absence of different concentrations of Mab B7-24 (FIG. 8A) or BB-1 (FIG. 8B). Proliferation in the absence of **antibody** ranged from 15,000 to 30,000 in primary MLC and from 20,000 to 60,000 in secondary MLC. Data shown are the. . .

US PAT NO: 5,741,899 [IMAGE AVAILABLE] L3: 7 of 24

DRAWING DESC:

DRWD(58)

In . . . T cell antigen receptor with the peptide antigen-MHC complex. The second costimulatory signal is provided through the interaction of the **CD28** or CTLA4 proteins on the T cell surface with either the B7-2 or **B7** proteins, their counterreceptors on the APC

(Clark and Ledbetter, Nature, 367:425-428 (1994); Croft, Current Opinion in Immunology, 6:431-437 (1994)). In. . . still maintaining antigen specificity. This chimeric receptor will link an ECD which is an antigen binding moiety such as an **antibody** or a viral receptor (e.g., CD4, the receptor for HIV) to a proliferation signaling domain which is a component of. . .

US PAT NO: 5,723,127 [IMAGE AVAILABLE] L3: 8 of 24

DETDESC:

DETD(66)

mAb OKT3-(IgG2a, anti-CD3) and OKM1-(IgG2b, anti-CD11b) producing cells were obtained from American Type Culture Collection (ATCC, Rockville, Md.); CK248 (IgM, anti-**CD28** [A. Moretta et al, J. Exp. Med., 162:823 (1985)] and 93 (IgG1, anti-CD25) were kindly provided by Dr. Lorenzo Moretta. . . Italy); B52.1 (IgM, anti-CD14) [B. Perussia et al, Blood, 59:382 (1982)] was produced in our laboratory. The mAb 9.3 (IgG2a, anti-**CD28** [J. Hansen et al, Immunogenetics, 10:247 (1980)] was donated by Drs. J. Hansen (Fred Hutchinson Cancer Center, Seattle, Wash.); mAb B7.4 (IgG, anti-**B7**) and mAb CD28.1, CD28.2, CD28.3, CD28.5, 15E8 (IgG1, anti-**CD28**) [J. Nunes et al, Int. Immunol., :5:311 (1992)] were obtained through the participation in the 5th International Conference on Human Leucocyte Differentiation Antigens. The specificity of all the anti-**CD28 antibodies** used was assigned at the Conference Workshop and confirmed in our laboratory by reactivity in immunofluorescence with **CD28**-transfected J32 cell line, but not with the **CD28**-negative parental cell lines. In immunofluorescence, mAb 9.3 inhibits the binding of mAb CK248 to **CD28**.sup.+ T cells. Anti-IL-2 polyclonal goat antiserum was prepared in our laboratory and, at 1:200 dilution, completely neutralizes the activity of .gtoreq.100 U/ml of IL-2. CTLA-4Ig [P. S. Linsley et al, J. Exp. Med., 174:561 (1991)] and **B7**-transfected CHO cells [P. S. Linsley et al, J. Exp. Med., 173:721 (1991)]were kindly provided by Dr. Peter S. Linsley (Bristol-Meyers Squibb Pharmaceutical Research Institute, Seattle, Wash.); **B7**-transfected L cells [E. E. Murphy et al, J. Exp. Med., in press (1993)] by Dr. Lewis Lanier (DNAX Research Institute,. . .

DETDESC:

DETD(82)

PHA blasts were incubated with the anti-**CD28 antibody** CK248 (ascites 0.2%) or with different numbers of L-cells transfected or not with the **B7** antigen in the presence or not of IL-12 (1 ng/ml). .sup.3 H-TdR uptake (cpm) was evaluated at 3 days after. . .

DETDESC:

DETD(86)

The results illustrated in FIG. 5 are mean.+-.S.E. of 4 experiments. IL-12 synergizes with stimulation of **CD28** by **antibodies** or by its ligand **B7** in inducing production of IFN-.gamma. from PHA-blasts. Control parental CHO cells or control ascites (not shown) had no significant effect. identical results were obtained with **B7**-transfected L cells (not shown).

DETDESC:

DETD(93)

The . . . of experiments summarized above provided results which demonstrate that, in the presence of an appropriate co-stimulus, i.e.,

signaling through the **CD28** receptor induced by **B7**-transfected cells or certain anti-**CD28 antibodies**, IL-12 can induce powerful and prolonged proliferation in activated T cells. Not only were the maximal levels of proliferation obtained. . . effective at concentrations 100- to 1000-fold lower than effective concentrations of IL-2. Moreover, the synergistic proliferation induced by IL-12 and anti-**CD28 antibody** was also observed with freshly isolated PBL. On both freshly isolated PBL or activated PHA-blasts, anti-**CD28 antibodies** or **B7**-transfected cells also synergized with IL-12 in inducing cytokine production.

US PAT NO: 5,718,883 [IMAGE AVAILABLE] L3: 9 of 24

SUMMARY:

BSUM(3)

Normal . . . T cell receptor (TCR)/CD3 complex. Recent data suggests that a second stimulation signal is generated through the T cell receptor **CD28** and corresponding ligands, such as the surface molecule **B7**. June et al., Immunol. Today, 11:211-216 (1990); and Harding et al., Nature, 356:607-609 (1992). Under normal circumstances **B7** is expressed on activated B and T lymphocytes, macrophages, and other antigen presenting cells. Freeman et al., J. Immunol., 143:2714-2722. . . (1993), also called B70 (Azuma et al., Nature, 366:76-79 (1993)) has also been found only on specialized APCs. Normally, the **B7** family of receptors are not expressed on cells that express only MHC class I molecules. Recently, thyroid cells from patients with autoimmune Graves' thyroiditis were reported to reveal specific anti-**B7** (B7-1) immunostaining while thyroid cells from normal individuals did not stain (Garcia-Cozar et al., Immunologia, 12:32 (abstract) (1993)). In addition, others have found that psoriatic but not unaffected skin keratinocytes stain with the BB-1 **antibody** which stains a **B7**-like molecule (Nickoloff et al., Am. J. Pathol., 142:1029-1040 (1993)). These reports indicate that cells other than conventional APCs can express **B7**-like molecules. More importantly, these studies have shown that epithelial cells do express a **B7**-like molecule in some T cell mediated autoimmune states.

DETDESC:

DETD(21)

The second class of assays, which determine **CD28** receptor-mediated signal transduction, can be carried out in a number of ways. For example, a T cell proliferation assay is. . . purified T cells, which have been exposed to an antigen, or submitogenic amounts of phorbol 12-myristate 13-acetate (PMA) or immobilized **antibodies** to CD3, to provide the first stimulatory signal, and to cells modified to express a potential **CD28** ligand. If a suitable **CD28** ligand, such as a **B7** polypeptide, is present on the modified cell, then the second, co-stimulatory signal will be delivered to the T cell, and. . . occur. As a control, in a second test, the T cells are first exposed to univalent (Fab) fragment of a **CD28** monoclonal **antibody**, and then exposed to the potential **CD28** ligand expressing cell. As described in Damle et al., J. Immunol., 140:1753-61 (1988); and in Gross et al., Nature, 356:607 (1992), co-stimulatory activity through **CD28** is not elicited by **CD28** univalent (Fab) fragments. Therefore, costimulatory effects of, e.g., **B7**, are prevented under these conditions.

DETDESC:

DETD(23)

A third assay method to determine **CD28** receptor-mediated signal

transduction can be carried out by screening for the effect of a potential **CD28** ligand on tyrosine phosphorylation. Vandenberghe et al., J. Exp. Med., 175:951 (1992), which is incorporated herein by reference, shows that the **CD28** ligand **B7** increases the tyrosine phosphorylation of a certain unknown 100 kDa substrate, and that this increase is prevented by pretreatment of the antigen-presenting cells that express **B7** with an anti-**B7 antibody**. This same test can be carried out using a cell modified to express a potential **CD28** ligand, and a corresponding **antibody**.

DETDESC:

DETD(27)

Once . . . slices of specific tissue, e.g., pancreatic tissue, are obtained from a transgenic animal and a non-transgenic littermate and stained with anti-**CD28** ligand **antibody**, such as the BB-1 monoclonal **antibody** (BB-1 mAb) directed against the human BB-1 molecule, Yokochi et al., J. Immunol., 128:823-827 (1981), or an anti-murine **B7 antibody**, Reiser et al., PNAS, USA, 89:271-275 (1992), to demonstrate tissue-specific **CD28** ligand expression. Other **CD28** ligands, for which **antibodies** have not yet been prepared, are likely to be discovered and cloned. These ligands also fall within the scope of. . .

DETDESC:

DETD(55)

Generally accepted symptoms of the onset or progression of Type 1 diabetes in animals include glycosuria, hyperglycemia, and weight loss. Insulin promoter-**B7** transgenic animals and non-transgenic but syngeneic animals are weighed weekly in the presence or absence of a potential therapeutic agent. . . II strips (Boehringer Mannheim, Indianapolis, Ind.), which require only one drop of blood per sample. The potential therapeutic agents, e.g., **antibodies**, peptides, or small molecules that block the interaction between the **CD28** receptor and its ligands, can be administered by injection, e.g., intravenous, intraarterial, subcutaneous, or intramuscular, or by intradermal or oral. . .

US PAT NO: 5,712,149 [IMAGE AVAILABLE] L3: 10 of 24

SUMMARY:

BSUM(14)

In contrast, lack of **CD28** co-stimulation at the time of antigen encounter (or CD3/TCR stimulation) results in anergy, a state of specific un-responsiveness. **Antibodies** to **B7** or soluble ligands such as CTLA-Ig (Linsley et al. J. Exp. Med. 174:561-569 (1991)) have been used in vitro and. . . rejection (Thompson et al., supra; Fraser and Weiss, supra; and Fraser et al., supra) by interfering with the interaction between **B7** and **CD28** (Johnson and Jenkins, supra; Schwartz, R. H., Cell 71:1065-1068 (1992); Linsley and Ledbetter, supra). **Antibodies** to **CD28** can substitute for co-stimulation by APCs in inducing immune responses and protecting T cells from anergy (Johnson and Jenkins, supra,. . .

DETDESC:

DETD(99)

The . . . nodular tumors when introduced into syngeneic mice (Chen et al., J. Exp. Med. supra). EL-4 cells lack expression of the **B7** ligand

for **CD28** and do not elicit protective immunity except by repeated injection of large numbers of irradiated tumor cells (ibid.). In contrast, EL-4 cells transduced to express **B7** are unable to form tumors in syngeneic mice. Instead, injection of mice with EL-4/**B7** cells causes the regression of existing EL-4 tumors and confers lasting protective immunity against subsequent injections with **B7**.sup.- EL-4 cells (ibid.). The ability of **CD28**-based chimeric receptors to augment the immune response to a relatively non-immunogenic tumor is tested in vivo using mice which are. . . whose lymphocytes express high levels of the CH28-3 receptor were identified by analyzing blood and lymphoid tissue by FACS with **antibodies** to human CD4. Such mice will be injected with non-manipulated EL-4 cells or EL-4 cells expressing the HIV envelope protein.. . . of tumors. The non-manipulated EL-4 cells should form tumors in both kinds of mice. These experiments demonstrate the ability of **CD28**-based chimeric receptors to provide co-stimulation in a situation in which antigen-responsive cells are present, but the natural immune response is. . .

US PAT NO: 5,707,624 [IMAGE AVAILABLE] L3: 11 of 24

DETDESC:

DETD(83)

Two KS lines were tested at least three times using an IFN-F concentration that induces **B7** on monocytes and ECs. In all tests, no **B7** was expressed by either KS line, despite induction of HLA-DR, and increased expression of ICAM-1 compared to non-IFN-.gamma. treated KS cells. Two different reagents were used to measure **B7** expression, a mouse anti-human **B7** (IgG1) **antibody** prepared by Repligen Corp. (Boston, Mass.) and an even more sensitive reagent, the human chimera fusion protein CTLA4-Ig, kindly provided by Peter Linsley (Seattle, Wash.). This fusion protein binds **B7** with a Kd of 12 nM, approximately 20-fold greater than the avidity of the interaction between **B7** and a **CD28** Ig fusion protein. The specificity of both the Repligen anti-**B7** mAB, and CTLA4-1g was confirmed by using CHO cells either mock transfected or **B7**-transfected, as well as by immunoprecipitation of .sup.125 I labeled cell lines expressing **B7** mRNA. Such a cell line is M16B cells that has successfully detected all 4 mRNA transcripts for **B7** (Savage, C. O. et al., Cell Immunol. 137:150-159 (1991)). Immunostaining 5 different KS cells revealed focal **B7** expression epidermal Langerhans cells, ECs, and dermal dendrocytes, but not KS tumor cells. Using these reagents, it was observed that unlike cultured ECs that upregulate **B7** expression following IFN-.gamma. (500 U/ml, 48 hrs), as well as ICAM-1 and HLA-DR, KS tumor cells only express ICAM-1 and HLA-DR, but not **B7**.

US PAT NO: 5,691,135 [IMAGE AVAILABLE] L3: 12 of 24

DETDESC:

DETD(9)

Yet . . . the compound is an immunosuppressive agent, most preferably a kinase inhibitor or cyclosporine. Alternatively, the compound is preferably an anti-receptor **antibody** which binds to a membrane protein. Even more preferably, the **antibody** binds to one of the following antigens: **B7**, **CD28**, CD72 or CD5. Still even more preferably, the compound is an anticytokine **antibody**, most preferably IL-6 or TGF-.beta..

DETDESC:

DETD(221)

Immunoglobulin . . . use of immunosuppressive agents such as kinase inhibitors or cyclosporine that interferes with intracellular signalling or lymphocyte cell physiology; anti-receptor **antibodies** against membrane proteins for T-B interaction (eg., **B7**, **CD28**, CD72, CD5); anti-cytokine **antibodies** against IL-6 or IL-2; and use of immunoglobulin-suppressive cytokines (eg., TGF-beta).

US PAT NO: 5,686,281 [IMAGE AVAILABLE] L3: 13 of 24

SUMMARY:

BSUM(14)

In contrast, lack of **CD28** co-stimulation at the time of antigen encounter (or CD3/TCR stimulation) results in anergy, a state of specific un-responsiveness. **Antibodies** to **B7** or soluble ligands such as CTLA-Ig (Linsley et al. J. Exp. Med. 174:561-569 (1991)) have been used in Vitro and. . . rejection (Thompson et al., supra; Fraser and Weiss, supra; and Fraser et al., supra) by interfering with the interaction between **B7** and **CD28** (Johnson and Jenkins, supra; Schwartz, R. H., Cell 71:1065-1068 (1992); Linsley and Ledbetter, supra). **Antibodies** to **CD28** can substitute for co-stimulation by APCs in inducing immune responses and protecting T cells from anergy (Johnson and Jenkins, supra,. . .

DETDESC:

DETD(99)

The . . . nodular tumors when introduced into syngeneic mice (Chen et al., J. Exp. Med. supra). EL-4 cells lack expression of the **B7** ligand for **CD28** and do not elicit protective immunity except by repeated injection of large numbers of irradiated tumor cells (ibid.). In contrast, EL-4 cells transduced to express **B7** are unable to form tumors in syngeneic mice. Instead, injection of mice with EL-4/**B7** cells causes the regression of existing EL-4 tumors and confers lasting protective immunity against subsequent injections with **B7**.sup.31 EL-4 cells (ibid.). The ability of **CD28**-based chimeric receptors to augment the immune response to a relatively non-immunogenic tumor is tested in vivo using mice which are. . . whose lymphocytes express high levels of the CH28-3 receptor were identified by analyzing blood and lymphoid tissue by FACS with **antibodies** to human CD4. Such mice will be injected with non-manipulated EL-4 cells or EL-4 cells expressing the HIV envelope protein.. . . of tumors. The non-manipulated EL-4 cells should form tumors in both kinds of mice. These experiments demonstrate the ability of **CD28**-based chimeric receptors to provide co-stimulation in a situation in which antigen-responsive cells are present, but the natural immune response is. . .

US PAT NO: 5,684,136 [IMAGE AVAILABLE] L3: 14 of 24

DETDESC:

DETD(74)

Chimeras . . . Cell. Biol. 110, 2221-2229 (1990); Watson et al., Nature 349, 164-167 (1991)); CD44* (Aruffo et al., Cell 61, 1303-1313 (1990)); **CD28*** and **B7*** (Linsley et al., J. Exp. Med. 173, 721-730 (1991)); CTLA-4* (Lisley et al., J. Expo. Med. 174, 561-569 (1991)); CD22*. . . they are, however, all common in that they can possess many of the desired chemical and biological properties of human **antibodies**.

US PAT NO: 5,652,224 [IMAGE AVAILABLE] L3: 15 of 24

DETDESC:

DETD(76)

Alternatively, . . . CD40 ligand on the T helper cell to the CD40 antigen on the B cell, and the binding of the **CD28** and/or CTLA4 ligands on the T cell to the **B7** antigen on the B cell. Without both interactions, the B cell cannot be activated to induce production of the neutralizing **antibody**.

DETDESC:

DETD(78)

Alternatively, an agent which blocks the **CD28** and/or CTLA4 ligands present on T helper cells interferes with the normal binding of those ligands with the antigen **B7** on the B cell. Thus, a soluble form of **B7** or an **antibody** to **CD28** or CTLA4, e.g., CTLA4-Ig [available from Bristol-Myers Squibb Co; see, e.g., European patent application 606,217, published Jul. 20, 1994] can. . .

US PAT NO: 5,637,481 [IMAGE AVAILABLE] L3: 16 of 24

DETDESC:

DETD(14)

The DNA sequence so replaced in the modified expression vector may encode any variable region of any **antibody** or other receptor. For example, the DNA sequence may encode the variable region or regions of an **antibody** which recognizes and binds the BR96 antigen, CD3, L6, **CD28**, CTLA4, or **B7**. Additionally, the DNA sequence may encode variable regions capable of binding to other cell surface antigens. Alternatively, the binding domains. . .

DETDESC:

DETD(42)

Additionally, . . . is provided which comprises a recombinant bispecific single chain cassette comprising a DNA sequence encoding a variable region(s) of any **antibody** and a DNA sequence encoding a ligand. Examples of such ligands include but are not limited to **B7**, CTLA4, **CD28**, CD40, CD3. Other examples of ligands include any of the leucocyte antigens.

DETDESC:

DETD(113)

Adhesion assays were performed essentially as described (Linsley, P. S., Clark, E. A., Ledbetter, J. A. (1990) T-cell antigen **CD28** mediates adhesion with B cells by interacting with activation antigen **B7**/BB1. Proc. Natl. Acad. Sci. USA 87:5031-5035), in the presence of 10 mM EDTA. Jurkat cells were first labeled with .sup.51 Cr and incubated with **antibody** stimuli, washed, and incubated with H2981 tumor cells and were examined microscopically. To prevent nonspecific binding to H2981 cells, the Jurkats and H2981 monolayers were incubated with an irrelevant **antibody** to saturate Fc receptors prior to addition of the CD3Ig (also referred to herein as CD3FvIg), L6Ig (also referred to herein as L6FvIg), or CD3-L6Ig (also referred to herein as CD3-L6FvIg) **antibody** derivatives. After the adhesion reactions were complete, monolayers were washed five times with ice-cold RPMI media, solubilized by addition of. . .

US PAT NO: 5,633,234 [IMAGE AVAILABLE] L3: 17 of 24

DETDESC:

DETD(61)

Many auto-immune diseases show a correlation with certain MHC class II haplotypes and are associated with aberrant auto-**antibody** production, suggesting that the generation of self-reactive MHC class II restricted CD4.sup.+ T cells is an important pathogenetic step. Given. . . anergized by engagement of their T cell receptor in the absence of a second signal (such as the co-engagement of **CD28** by its ligand **B7**), it follows that the efficient presentation of an MHC class II restricted antigen on an MHC class II.sup.+ cell that. . .

US PAT NO: 5,580,756 [IMAGE AVAILABLE] L3: 18 of 24

ABSTRACT:
The invention identifies the **B7** antigen as a ligand that is reactive with the **CD28** receptor on T cells. Fragments and derivatives of the **B7** antigen and **CD28** receptor, including fusion proteins having amino acid sequences corresponding to the extracellular domains of **B7** or **CD28** joined to amino acid sequences encoding portions of human immunoglobulin C.gamma.1, are described. Methods are provided for using **B7** antigen, its fragments and derivatives, and the **CD28** receptor, its fragments and derivatives, as well as **antibodies** and other molecules reactive with **B7** antigen and/or the **CD28** receptor, to regulate **CD28** positive T cell responses, and immune responses mediated by T cells. The invention also includes an assay method for detecting. . .

SUMMARY:

BSUM(17)

Accordingly, the present invention identifies the **B7** antigen as a ligand recognized by the **CD28** receptor. The **B7** antigen, or its fragments or derivatives are reacted with **CD28** positive T cells to regulate T cell interactions with other cells. Alternatively, the **CD28** receptor, its fragments or derivatives are reacted with **B7** antigen to regulate interactions of **B7** positive cells with T cells. In addition, **antibodies** or other molecules reactive with the **B7** antigen or **CD28** receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses.

SUMMARY:

BSUM(18)

A preferred embodiment of the invention provides a method for regulating **CD28** specific T cell interactions by reacting **CD28** positive T cells with **B7** antigen, or its fragments or derivatives, so as to block the functional interaction of T cells with other cells. The method for reacting a ligand for **CD28** with T cells may additionally include the use of anti-CD monoclonal **antibodies** such as anti-CD2 and/or anti-CD3 monoclonal **antibody**.

DETDESC:

DETD(4)

Recently, . . . al., (J. Immunol. 143(8):2714-2722 (1989)) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by monoclonal **antibody** (mAb) **B7** (Freedman et al., J. Immunol. 139:3260 (1987)). COS cells transfected with this cDNA were

shown to stain by both mAb **B7** and mAb BB-1 (Clark et al., Human Immunology 16:100-113 (1986), and Yokochi et al., (1981), supra; Freeman et al., (1989) supra; and Freedman et al., (1987), supra)). The ligand for **CD28** was identified by the experiments described herein, as the **B7**/BB-1 antigen isolated by Freeman et al., (Freedman et al., and Freeman et al., supra, both of which are incorporated by. . .

DETDESC:

DETD(8)

The term "derivative" also includes monoclonal **antibodies** reactive with the **B7** antigen or **CD28** receptor, or fragments thereof, and **antibodies** reactive with the B7Ig and CD28Ig fusion proteins of the invention.

DETDESC:

DETD(27)

In addition to the fusion proteins of the invention, monoclonal **antibodies** reactive with the **B7** antigen and **CD28** receptor, and reactive with B7Ig and CD28Ig fusion proteins, may be produced by hybridomas prepared using known procedures, such as. . .

DETDESC:

DETD(28)

These techniques involve the use of an animal which is primed to produce a particular **antibody**. The animal can be primed by injection of an immunogen (e.g. the B7Ig fusion protein) to elicit the desired immune response, i.e. production of **antibodies** reactive with the ligand for **CD28**, the **B7** antigen, from the primed animal. A primed animal is also one which is expressing a disease. Lymphocytes derived from the lymph nodes, spleens or peripheral blood of primed, diseased animals can be used to search for a particular **antibody**. The lymphocyte chromosomes encoding desired immunoglobulins are immortalized by fusing the lymphocytes with myeloma cells, generally in the presence of. . .

DETDESC:

DETD(32)

In addition, fragments of these **antibodies** containing the active binding region of the extracellular domain of **B7** or **CD28** antigen, such as Fab, F(ab').sub.2 and Fv fragments, may be produced. Such fragments can be produced using techniques well established. . .

DETDESC:

DETD(36)

It is expected that administration of the **B7** antigen will result in effects similar to the use of anti-**CD28** monoclonal **antibodies** (mAbs) reactive with the **CD28** receptor in vivo. Thus, because anti-**CD28** mAbs may exert either stimulatory or inhibitory effects on T cells, depending, in part, on the degree of crosslinking or "aggregation" of the **CD28** receptor (Damle, J. Immunol. 140:1753-1761 (1988); Ledbetter et al., Blood 75(7):1531-1539 (1990)) it is expected that the **B7** antigen, its fragments and derivatives, will act to stimulate or inhibit T cells in a manner similar to the effects observed for an anti-**CD28** monoclonal **antibody**, under similar conditions in vivo. For example, administration of **B7** antigen, e.g. as a soluble B7Ig fusion protein to react with **CD28** positive T cells, will bind

the **CD28** receptor on the T cells and result in inhibition of the functional responses of T cells. Under conditions where T cell interactions are occurring as a result of contact between T cells and B cells, binding of introduced **B7** antigen in the form of a fusion protein that binds to **CD28** receptor on **CD28** positive. T cells should interfere, i.e..inhibit, the T cell interactions with B cells. Likewise, administration of the **CD28** antigen, or its fragments and derivatives in vivo, for example in the form of a soluble CD28Ig fusion protein, will result in binding of the soluble CD28Ig to **B7** antigen, preventing the endogenous stimulation of **CD28** receptor by **B7** positive cells such as activated B cells, and interfering with the interaction of **B7** positive cells with T cells.

DETDESC:

DETD(40)

In an additional embodiment of the invention, other reagents, such as molecules reactive with **B7** antigen or the **CD28** receptor are used to regulate T and/or B cell responses. For example, **antibodies** reactive with the CD28Ig fusion proteins, and Fab fragments of CD28Ig, may be prepared using the CD28Ig fusion protein as immunogen, as described above. These anti-**CD28 antibodies** may be screened to identify those capable of inhibiting the binding of the **B7** antigen to **CD28** antigen. The **antibodies** or **antibody** fragments such as Fab fragments may then be used to react with the T cells, for example, to inhibit **CD28** positive T cell proliferation. The use of Fab fragments of the 9.3 monoclonal **antibody**, or Fab fragments of the anti-CD28Ig monoclonal **antibodies** as described herein, is expected to prevent binding of **CD28** receptor on T cells to **B7** antigen, for example on B cells. This will result in inhibition of the functional response of the T cells.

DETDESC:

DETD(41)

Similarly, anti-**B7** monoclonal **antibodies** such as BB-1 mAb, or anti-B7Ig monoclonal **antibodies** prepared as described above using B7Ig fusion protein as immunogen, may be used to react with **B7** antigen positive cells such as B cells to inhibit B cell interaction via the **B7** antigen with **CD28** positive T cells.

DETDESC:

DETD(47)

Under some circumstances, as noted above, the effect of administration of the **B7** antigen, its fragments or derivatives in vivo is stimulatory as a result of aggregation of the **CD28** receptor. The T cells are stimulated resulting in an increase in the level of T cell cytokines, mimicking the effects of T cell/B cell contact on triggering of the **CD28** antigen on T cells. In other circumstances, inhibitory effects may result from blocking by the **B7** antigen of the **CD28** triggering resulting from T cell/B cell contact. For example, the **B7** antigen may block T cell proliferation. Introduction of the **B7** antigen in vivo will thus produce effects on both T and B cell mediated immune responses. The ligand may also. . . subject in combination with the introduction of cytokines or other therapeutic reagents. Alternatively, for cancers associated with the expression of **B7** antigen, such as **B7** lymphomas, carcinomas, and T cell leukemias, ligands reactive with the **B7** antigen, such as anti-B7Ig monoclonal **antibodies**, may be used to inhibit the function of malignant B cells.

DETDESC:

DETD(54)

The results presented herein also demonstrate that **antibodies** reactive with **CD28** and **B7** antigen specifically block helper T.sub.h -mediated immunoglobulin production by allogeneic B cells, providing evidence of the role of **CD28/B7** interactions in the collaboration between T and B cells.

DETDESC:

DETD(100)

Cells . . . obtained from the ATCC and ascitic fluids from these hybridomas were generated in pristane-primed BALB/c mice. Production and characterization of anti-**CD28** mAb 9.3 (IgG2a) has been described by Ledbetter et al., J. Immunol. 135:2331 (1985); Hara et al., J. Exp. Med.. . . described by Damle and Doyle, J. Immunol 143:1761 (1989), incorporated by reference herein, was provided by Dr. Engleman and mAb anti-**B7 antibody** (BB1; IgM) as described by Tokochi et al., J. Immunol. 128:823 (1981), incorporated by reference herein, was provided by Dr.. . .

DETDESC:

DETD(176)

The above results demonstrate that the ligand for **CD28** receptor, the **B7** antigen, is expressed on activated B cells and cells of other lineages. These results also show that **CD28** receptor and its ligand, **B7**, play a pivotal role during both the activation of CD.sub.4.sup.+ T.sub.h cell and T.sub.h -induced differentiation of B cells. The inhibition of anti-**CD28** and anti-**B7** mAbs on the cognate T.sub.h :B interaction also provide the basis for employing the CD28Ig and B7Ig fusion proteins, and monoclonal **antibodies** reactive with these proteins, to treat various autoimmune orders associated with exaggerated B cell activation such as insulin-dependent diabetes mellitus,. . .

US PAT NO: 5,576,423 [IMAGE AVAILABLE] L3: 19 of 24

SUMMARY:

BSUM(4)

The . . . N.Y. Increased adhesion between T cells and antigen presenting cells (APC) or other forms of primary stimuli, e.g., immobilized monoclonal **antibodies** (mAb), can potentiate the T-cell receptor signals. T-cell activation and T cell expansion depends upon engagement of the T-cell receptor. . . (1990) Immunol. Today 11:211-216; and Jenkins (1994) Immunity 1:443-446. A major, and well-studied, co-stimulatory interaction for T cells involves either **CD28** or CTLA-4 on T cells with either **B7** or B70 (Jenkins (1994) Immunity 1:443-446). Recent studies on **CD28** deficient mice (Shahinian, et al. (1993) Science 261:609-612; Green, et al. (1994) Immunity 1:501-508) and CTLA-4 immunoglobulin expressing transgenic mice. . .

US PAT NO: 5,571,515 [IMAGE AVAILABLE] L3: 20 of 24

DETDESC:

DETD(67)

mAb OKT3-(IgG2a, anti-CD3) and OKM1-(IgG2b, anti-CD11b) producing cells were obtained from American Type Culture Collection (ATCC, Rockville,

Md.); CK248 (IgM, anti-**CD28** [A. Moretta et al, J. Exp. Med., 162:823 (1985)] and 93 (IgG1, anti-CD25) were kindly provided by Dr. Lorenzo Moretta. . . Italy); B52.1 (IgM, anti-CD14) [B. Perussia et al, Blood, 59.:382 (1982)] was produced in our laboratory. The mAb 9.3 (IgG2a, anti-**CD28** [J. Hansen e tal, Immunogenetics, 10:247 (1980)] was donated by Drs. J. Hansen (Fred Hutchinson Cancer Center, Seattle, Wash.); mAb B7.4 (IgG, anti-**B7**) and mAb CD28.1, CD28.2, CD28.3, CD28.5, 15E8 (IgG1, anti-**CD28**) [J. Nunes et al, Int. Immunol., 5:311 (1992)] were obtained through the participation in the 5th International Conference on Human Leucocyte Differentiation Antigens. The specificity of all the anti-**CD28 antibodies** used was assigned at the Conference Workshop and confirmed in our laboratory by reactivity in immunofluorescence with **CD28**-transfected J32 cell line, but not with the **CD28**-negative parental cell lines. In immunofluorescence, mAb 9.3 inhibits the binding of mAb CK248 to **CD28**.sup.+ T cells. Anti-IL-2 polyclonal goat antiserum was prepared in our laboratory and, at 1:200 dilution, completely neutralizes the activity of .gtoreq.100 U/ml of IL-2. CTLA-4Ig [P. S. Linsley et al, J. Exp. Med., 174:561 (1991)] and **B7**-transfected CHO cells [P. S. Linsley et al, J. Exp. Med., 173:721 (1991)] were kindly provided by Dr. Peter S. Linsley (Bristol-Meyers Squibb Pharmaceutical Research Institute, Seattle, Wash.); **B7**-transfected L cells [E. E. Murphy et al, J. Exp. Med., in press (1993)] by Dr. Lewis Lanier (DNAX Research Institute,. . .

DETDESC:

DETD(83)

PHA blasts were incubated with the anti-**CD28 antibody** CK248 (ascites 0.2%) or with different numbers of L-cells transfected or not with the **B7** antigen in the presence or not of IL-12 (1 ng/ml). .sup.3 H-TdR uptake (cpm) was evaluated at 3 days after. . .

DETDESC:

DETD(87)

The results illustrated in FIG. 5 are mean.+-.S.E. of 4 experiments. IL-12 synergizes with stimulation of **CD28** by **antibodies** or by its ligand **B7** in inducing production of IFN-.gamma. from PHA-blasts. Control parental CHO cells or control ascites (not shown) had no significant effect. Identical results were obtained with **B7**-transfected L cells (not shown).

DETDESC:

DETD(88)

I. Synergy Between Anti-**CD28 Antibody** CK248 and **B7**-Transfected CHO-cells With IL-12 in Inducing Cytokine (IFN-.gamma., TNF-.alpha., and GM-CSF) Production From PHA-Blasts

DETDESC:

DETD(94)

The . . . of experiments summarized above provided results which demonstrate that, in the presence of an appropriate co-stimulus, i.e., signaling through the **CD28** receptor induced by **B7**-transfected cells or certain anti-**CD28 antibodies**, IL-12 can induce powerful and prolonged proliferation in activated T cells. Not only were the maximal levels of proliferation obtained. . . effective at concentrations 100- to 1000-fold lower than effective concentrations of IL-2. Moreover, the synergistic proliferation induced by IL-12 and anti-**CD28 antibody** was also observed with freshly isolated PBL.

On both freshly isolated PBL or activated PHA-blasts, anti-**CD28** **antibodies** or **B7**-transfected cells also synergized with IL-12 in inducing cytokine production.

US PAT NO: 5,525,503 [IMAGE AVAILABLE] L3: 21 of 24

SUMMARY:

BSUM(6)

The natural ligand for **CD28** has been identified as **B7**/BB1 (Linsley et al., 1990, Proc. Natl. Acad. Sci. USA 87:5031). **B7** is a surface glycoprotein that is expressed on activated B cells and interferon-.gamma. treated monocytes (Freeman et al., 1989, J.. . . Immunol. 143:2714; Yockochi et al., 1982, J. Immunol. 128 823; Freedman et al., 1991, Cell. Immunol. 137:429). The binding of **B7**/BB1 to **CD28** potentiates the level of proliferation initiated by the antigen receptor complex (Koulova et al., 1991, J. Exp. Med. 173:759; Linsley;. . . Sci. USA 88:6575). Similarly, the inability of fixed accessory cells to induce T-cell response can be corrected by ligation of **CD28** with allogeneic accessory cells or **antibody** (Jenkins et al., 1988, J. Immunol. 140:3324; Harding). Engagement of the TcR.zeta./CD3 complex in the absence of **CD28** ligation leads to a state of anergy. The requirement for this second signal may play an important role in vivo. . .

DETDESC:

DETD(40)

The importance of uncovering the signalling mechanism of **CD28** in T cells is underlined by its importance in immunotherapy against tumor cells, and in autoimmunity. The generation of CD8+ cytotoxic T cells against tumors is greatly amplified by the expression of **B7** in target cells. For example, melanoma cells, normally resistant to cytotoxic killing, are killed when transfected with the **CD28** ligand, **B7** (Chen et al., supra). Similarly, **B7** expression on Langerhans cells induces T-cell infiltration, MHC class II recognition and diabetes in transgenic mice. The mechanism involves the direct activation of CD8+ cells via IL-2, and can be blocked by anti-**B7** **antibody** binding to the **B7** ligand, **CD28**. Stimulation of PI 3-kinase activity through the **B7**/**CD28** signalling mechanism is the likely intracellular messenger responsible for the enhanced generation of cytotoxic T cells and/or their eventual effector. . .

DETDESC:

DETD(74)

The assay utilizes a cell that expresses PI 3-kinase and **CD28**. The cell is most preferably a T cell such as HPB-ALL or Jurkat, but may be any type of cell which expresses **CD28** on its surface and PI 3-kinase in its cytoplasm, e.g., a cell transfected with cDNAs encoding **CD28** and/or PI 3-kinase. A sample of cells is incubated in the presence or in the absence of a candidate compound.. . . to the pre-established standard as the control. Alternatively, controls could be run in parallel with each screening assay. Cell surface **CD28** is cross-linked with, e.g., a **CD28**-specific **antibody** or a **CD28** ligand, such as **B7**. The **CD28**-PI 3-kinase-complex is immunoprecipitated with Protein A Sepharose beads, subjected to SDS-PAGE under denaturing conditions, and immunoblotted with **antibody** specific for PI 3-kinase, e.g., an anti-p85 **antibody**. A reduction of the amount of protein on the immunoblot compared to a standard or to a control immunoblot carried out in the absence of a candidate compound, indicates inhibition of association of PI 3-kinase with **CD28**. The intensity of staining can

be quantitated by means of standard densitometric techniques.

US PAT NO: 5,521,288 [IMAGE AVAILABLE] L3: 22 of 24

ABSTRACT:
The invention identifies the **B7** antigen as a ligand that is reactive with the **CD28** receptor on T cells. Fragments and derivatives of the **B7** antigen and **CD28** receptor, including fusion proteins having amino acid sequences corresponding to the extracellular domains of **B7** or **CD28** joined to amino acid sequences encoding portions of human immunoglobulin C.gamma.1, are described. Methods are provided for using **B7** antigen, its fragments and derivatives, and the **CD28** receptor, its fragments and derivatives, as well as **antibodies** and other molecules reactive with **B7** antigen and/or the **CD28** receptor, to regulate **CD28** positive T cell responses, and immune responses mediated by T cells. The invention also includes an assay method for detecting. . .

SUMMARY:

BSUM(17)

Accordingly, the present invention identifies the **B7** antigen as a ligand recognized by the **CD28** receptor. The **B7** antigen, or its fragments or derivatives are reacted with **CD28** positive T cells to regulate T cell interactions with other cells. Alternatively, the **CD28** receptor, its fragments or derivatives are reacted with **B7** antigen to regulate interactions of **B7** positive cells with T cells. In addition, **antibodies** or other molecules reactive with the **B7** antigen or **CD28** receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses.

SUMMARY:

BSUM(18)

A preferred embodiment of the invention provides a method for regulating **CD28** specific T cell interactions by reacting **CD28** positive T cells with **B7** antigen, or its fragments or derivatives, so as to block the functional interaction of T cells with other cells. The method for reacting a ligand for **CD28** with T cells may additionally include the use of anti-CD monoclonal **antibodies** such as anti-CD2 and/or anti-CD3 monoclonal **antibody**.

DETDESC:

DETD(4)

Recently, . . . al., (J. Immunol. 143(8):2714-2722 (1989)) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by monoclonal **antibody** (mAb) **B7** (Freedman et al., J. Immunol. 139:3260 (1987)). COS cells transfected with this cDNA were shown to stain by both mAb **B7** and mAb BB-1 (Clark et al., Human Immunology 16:100-113 (1986), and Yokochi et al., (1981), supra; Freeman et al., (1989) supra; and Freedman et al., (1987), supra)). The ligand for **CD28** was identified by the experiments described herein, as the **B7**/BB-1 antigen isolated by Freeman et al., (Freedman et al., and Freeman et al., supra, both of which are incorporated by. . .

DETDESC:

DETD(8)

The term "derivative" also includes monoclonal **antibodies** reactive with the **B7** antigen or **CD28** receptor, or fragments thereof, and

antibodies reactive with the B7Ig and CD28Ig fusion proteins of the invention.

DETDESC:

DETD(27)

In addition to the fusion proteins of the invention, monoclonal **antibodies** reactive with the **B7** antigen and **CD28** receptor, and reactive with B7Ig and CD28Ig fusion proteins, may be produced by hybridomas prepared using known procedures, such as. . .

DETDESC:

DETD(28)

These techniques involve the use of an animal which is primed to produce a particular **antibody**. The animal can be primed by injection of an immunogen (e.g. the B7Ig fusion protein) to elicit the desired immune response, i.e. production of **antibodies** reactive with the ligand for **CD28**, the **B7** antigen, from the primed animal. A primed animal is also one which is expressing a disease. Lymphocytes derived from the lymph nodes, spleens or peripheral blood of primed, diseased animals can be used to search for a particular **antibody**. The lymphocyte chromosomes encoding desired immunoglobulins are immortalized by fusing the lymphocytes with myeloma cells, generally in the presence of. . .

DETDESC:

DETD(32)

In addition, fragments of these **antibodies** containing the active binding region of the extracellular domain of **B7** or **CD28** antigen, such as Fab, F(ab').sub.2 and Fv fragments, may be produced. Such fragments can be produced using techniques well established. . .

DETDESC:

DETD(36)

It is expected that administration of the **B7** antigen will result in effects similar to the use of anti-**CD28** monoclonal **antibodies** (mAbs).reactive with the **CD28** receptor in vivo. Thus, because anti-**CD28** mAbs may exert either stimulatory or inhibitory effects on T cells, depending, in part, on the degree of crosslinking or "aggregation" of the **CD28** receptor (Damle, J. Immunol. 140:1753-1761 (1988); Ledbetter et al., Blood 75(7):1531-1539 (1990)) it is expected that the **B7** antigen, its fragments and derivatives, will act to stimulate or inhibit T cells in a manner similar to the effects observed for an anti-**CD28** monoclonal **antibody**, under similar conditions in vivo. For example, administration of **B7** antigen, e.g. as a soluble B7Ig fusion protein to react with **CD28** positive T cells, will bind the **CD28** receptor on the T cells and result in inhibition of the functional responses of T cells. Under conditions where T cell interactions are occurring as a result of contact between T cells and B cells, binding of introduced **B7** antigen in the form of a fusion protein that binds to **CD28** receptor on **CD28** positive T cells should interfere, i.e. inhibit, the T cell interactions with B cells. Likewise, administration of the **CD28** antigen, or its fragments and derivatives in vivo, for example in the form of a soluble CD28Ig fusion protein, will result in binding of the soluble CD28Ig to **B7** antigen, preventing the endogenous stimulation of **CD28** receptor by **B7** positive cells such as activated B cells, and interfering with the interaction of **B7** positive cells with T cells.

DETDESC:

DETD(40)

In an additional embodiment of the invention, other reagents, such as molecules reactive with **B7** antigen or the **CD28** receptor are used to regulate T and/or B cell responses. For example, **antibodies** reactive with the CD28Ig fusion proteins, and Fab fragments of CD28Ig, may be prepared using the CD28Ig fusion protein as immunogen, as described above. These anti-**CD28 antibodies** may be screened to identify those capable of inhibiting the binding of the **B7** antigen to **CD28** antigen. The **antibodies** or **antibody** fragments such as Fab fragments may then be used to react with the T cells, for example, to inhibit **CD28** positive T cell proliferation. The use of Fab fragments of the 9.3 monoclonal **antibody**, or Fab fragments of the anti-CD28Ig monoclonal **antibodies** as described herein, is expected to prevent binding of **CD28** receptor on T cells to **B7** antigen, for example on B cells. This will result in inhibition of the functional response of the T cells.

DETDESC:

DETD(41)

Similarly, anti-**B7** monoclonal **antibodies** such as BB-1 mAb, or anti-B7Ig monoclonal **antibodies** prepared as described above using B7Ig fusion protein as immunogen, may be used to react with **B7** antigen positive cells such as B cells to inhibit B cell interaction via the **B7** antigen with **CD28** positive T cells.

DETDESC:

DETD(47)

Under some circumstances, as noted above, the effect of administration of the **B7** antigen, its fragments or derivatives in vivo is stimulatory as a result of aggregation of the **CD28** receptor. The T cells are stimulated resulting in an increase in the level of T cell cytokines, mimicking the effects of T cell/B cell contact on triggering of the **CD28** antigen on T cells. In other circumstances, inhibitory effects may result from blocking by the **B7** antigen of the **CD28** triggering resulting from T cell/B cell contact. For example, the **B7** antigen may block T cell proliferation. Introduction of the **B7** antigen in vivo will thus produce effects on both T and B cell mediated immune responses. The ligand may also. . . subject in combination with the introduction of cytokines or other therapeutic reagents. Alternatively, for cancers associated with the expression of **B7** antigen, such as **B7** lymphomas, carcinomas, and T cell leukemias, ligands reactive with the **B7** antigen, such as anti-B7Ig monoclonal **antibodies**, may be used to inhibit the function of malignant B cells.

DETDESC:

DETD(54)

The results presented herein also demonstrate that **antibodies** reactive with **CD28** and **B7** antigen specifically block helper T.sub.h -mediated immunoglobulin production by allogeneic B cells, providing evidence of the role of **CD28/B7** interactions in the collaboration between T and B cells.

DETDESC:

DETD(100)

Cells . . . obtained from the ATCC and acidic fluids from these hybridomas were generated in pristane-primed BALB/c mice. Production and characterization of anti-**CD28** mAb 9.3 (IgG2a) has been described by Ledbetter et al., J. Immunol. 135:2331 (1985); Hara et al., J. Exp. Med.. . . described by Damle and Doyle, J. Immunol 143:1761 (1989), incorporated by reference herein, was provided by Dr. Engleman and mAb anti-**B7 antibody** (BB-1; IgM) as described by Tokochi et al., J. Immunol. 128:823 (1981), incorporated by reference herein, was provided by Dr.. . .

DETDESC:

DETD(176)

The above results demonstrate that the ligand for **CD28** receptor, the **B7** antigen, is expressed on activated B cells and cells of other lineages. These results also show that **CD28** receptor and its ligand, **B7**, play a pivotal role during both the activation of CD4.sup.+ T.sub.h cell and T.sub.h -induced differentiation of B cells. The inhibition of anti-**CD28** and anti-**B7** mAbs on the cognate T.sub.h :B interaction also provide the basis for employing the CD28Ig and B7Ig fusion proteins, and monoclonal **antibodies** reactive with these proteins, to treat various autoimmune orders associated with exaggerated B cell activation such as insulindependent diabetes mellitus,. . .

US PAT NO: 5,474,897 [IMAGE AVAILABLE] L3: 23 of 24

SUMMARY:

BSUM(7)

Weiss et al. (1986) J. Immunol. 137:819-825, describes the ability to activate purified T cells and Jurkat cells by exposure to anti-**CD28 antibodies** and certain T cell receptor stimulants. Durand et al. (1988) Mol. Cell. Biol. 8:1715-1724, describes the construction of pIL-2-Luc. Fraser et al. (1991) Science 251:313-316, describes the effects of **CD28** stimulation on IL-2 enhancer activity in Jurkat cells transiently transfected with pIL-2-Luc. Exposure of the transfected cells to anti-**CD28** and TCR stimuli resulted in enhanced luciferase activity. A ligand for stimulating the **CD28** receptor present on activated B cells (**B7**/BB1) is described in Lindsley et al. (1990) Proc. Natl. Acad. Sci. USA 87:5031-5035. The importance of the **CD28** costimulatory pathway is described in Fraser et al. (1992) J. Exp. Med. 175:1131-1134.

SUMMARY:

BSUM(11)

The . . . are obtained from a T cell line which stably incorporates a DNA sequence comprising an enhancer region responsive to a **CD28**-regulated nuclear binding protein, a promoter, and a marker gene. The enhancer region is derived from or normally associated with a. . . The T cells are activated through the T cell receptor (TCR) and are cultured under conditions selected to induce the **CD28** signal transduction pathway. Conveniently, TCR activation is mimicked by exposure to ionomycin and phorbol 12-myistate 13-acetate (PMA), or by stimulating cells with anti-TCR **antibody** or with mitogenic lectins. Induction of the **CD28** pathway may be achieved by exposure to anti-**CD28 antibody** to **B7** expressing antigen presenting cells, or to a soluble form of **B7** protein.

DETDESC:

DETD(13)

Once . . . expression of the marker gene. Such conditions require activation or stimulation of both the T cell receptor (TCR) and the **CD28** receptor. Stimulation of TCR can conveniently be provided by the addition of ionomycin at a concentration of about 0.5 .mu.M. . . by increasing the Ca.sup.2+ concentration and activating protein kinase C, respectively. Other techniques for activating TCR include stimulation with anti-TCR **antibodies**, with lectins such as concanavalin A or phytohemagglutinin, or with staphylococcal enterotoxins. Activation of the **CD28** receptor may be obtained by exposing the cells to anti-**CD28 antibody**. Suitable anti-**CD28 antibodies** can be obtained from hybridoma cell line MAb 9.3 (Hansen et al. (1980) Immunogenetics 10:247-252) or with other commercially available anti-**CD28 antibodies**, with **antibodies** typically being introduced at dilutions on the order of 1:10,000 of ascitic fluid or 10-1000 ng/ml of pure **antibody**. Other techniques for activating the **CD28** receptor include stimulation with cell lines expressing the **B7** antigen, with soluble **B7** protein, or with **B7** fusion proteins.

US PAT NO: 5,434,131 [IMAGE AVAILABLE] L3: 24 of 24

SUMMARY:

BSUM(13)

Expression . . . soluble derivatives of cell-surface glycoproteins in the immunoglobulin gene superfamily has been achieved for CD4, the receptor for HIV-1, and **CD28** and **B7** receptors, using hybrid fusion molecules consisting of DNA sequences encoding amino acids corresponding to portions of the extracellular domain of CD4 receptor fused to **antibody** domains (immunoglobulin .gamma.1 (Capon et al., Nature 337:525-531 (1989) (CD4) and Linsley et al., J. Exp. Med., supra (**CD28** and **B7**)).

DETDESC:

DETD(36)

The . . . is expected that CTLA4Ig will act to inhibit T cells in a manner similar to the effects observed for the anti-**CD28 antibody**, under similar conditions in vivo. Under conditions where T cell/B cell interactions are occurring as a result of contact between T cells and B cells, binding of introduced CTLA4Ig to react with **B7** antigen positive cells, for example B cells, may interfere, i.e. inhibit, the T cell/B cell interactions resulting in regulation of. . .

DETDESC:

DETD(46)

Anti-**B7** monoclonal **antibodies** prepared-as described above may be used to bind to **B7** antigen to inhibit interactions of **CD28**-positive or CTLA4-positive T cells with **B7** positive cells. Anti-CTLA4 monoclonal **antibodies** may be used to bind to CTLA4 receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

DETDESC:

DETD(88)

mAbs. Murine monoclonal **antibodies** (mAbs) 9.3 (anti-**CD28**) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-**B7** antigen) and rat mAb 187.1 (anti-mouse K chain) have been described previously (Ledbetter et

al., Proc. Natl. Acad. Sci. 84:1384-1388.

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art unit 1644
308-3997

1.mchugh et al.
Clinical Immunology and Immunopathology 87(1): 50 -59 (1998)

2. horspool et al. j. immunol. 160 (6) : 2706 -14 (1998)

3. fargeas et al. j. exp. med. 182 (3) : 667 -75 (1995)

4. turcovski-corrales et al. eur. j. immunol. 25 (11) : 3087 - 93 (1995)

5. krummel et al. j. exp. med. 182 (2) : 459 -65 (1995)

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5. krummel et al. j. exp. med. 182 (2) : 459 -65 (1995)

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2/K/1 (Item 1 from file: 654)
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... in vivo growth of the tumor cell line V51Blim10 in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28** . FIG. 1B is a graph illustrating the average tumor size in mice injected with 2X10...

...injected with V51Blim10 cells.

FIG. 2 is a graph showing the in vivo growth of **B7**-51BLim10 tumors in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28**.

FIG. 3 shows the rejection of wild-type colon carcinoma cells by mice previously treated with V51BLim10 cells and anti-**CTLA-4 antibody**.

FIG. 4 shows the growth of established tumors after treatment with anti-**CTLA-4 antibody**.

FIG. 5 shows the growth of the murine fibrosarcoma SA1N in the absence or presence of anti-**CTLA-4 antibodies**.

FIGS. 6A to 6E illustrate the adjuvant effect of anti-**CTLA-4 antibodies** in the response of T cells to peptide antigens.

FIGS. 7A to 7F illustrate the of CTLA-4/**B7** blockade on the proliferation of purified CD4+ T cells. In FIG. 8B, detection of IL... ...4Ig but not CD4Ig in ELISAs; 2) the ability to block CTLA-4Ig binding to **B7** transfectants; 3) the ... T cells but not freshly isolated T cells; and 4) the ability to stain a **CTLA-4** transfectant but not control transfectants.

The ability of **antibody** 9H10 to block CTLA-4Ig binding to **B7** transfectants was demonstrated as follows. Approximately 10 mu l of mAb 9H10 was incubated at... 10,000 live gated events were collected for analysis. The results showed that the 9H10 **antibody** blocked **CTLA-4** binding to **B7**-EL-4 cells.

The ability of the 9H10 antibody to stain activated T cells but...tumors were measured with calipers. All of the mice left untreated, or treated with anti-**CD28** antibody, developed progressively growing tumors and required euthanasia by 35 days after inoculation. In contrast, all mice treated with anti-**CTLA-4 antibody** completely rejected their tumors after a short period of limited growth. As shown in FIG...with calipers. The data from this experiment is shown in FIG. 2. Treatment with anti-**CTLA-4 antibodies** inhibited **B7**-51 BLim10 tumor growth as compared to the anti-**CD28** and control groups. All mice in the untreated and anti-**CD28** treated groups developed small tumors that grew progressively for five to ten days and then...in vitro and that signals through CTLA-4 inhibit the response. In vivo, blockade of **CD28** by FAb fragments or intact antibodies have the opposite effects upon V beta 8+ expansion to a similar blockade with anti-**CTLA-4** FAb fragments or intact **antibodies**. Analysis of the kinetics of the expansion imply that signals through **CD28** promote T cell expansion, whereas an opposing signal through CTLA-4 functions during T cell...96 well

round bottom plates. SEB was added at the indicated concentrations. Where indicated, anti-**CD28** was added at a 1:1000 dilution of ascites, anti-**B7-1** was added at 5 mu g/ml and anti-**B7**-2 was added at 20 mu g/ml, and equal quantities of non-specific control **antibody** 560.31 were added. For FAb experiments, anti- **CD28**, anti-**CTLA-4** or control FAb fragments were added at 100 mu g/ml. Cultures were incubated for...B7-1/B7-2 induced a slight but reproducible increase in proliferation compared to anti-**CD28** by itself, suggesting that another **B7** ligand besides **CD28** (i.e. CTLA-4) might be important in downregulating the response of T cells to SEB.

To address the relative contibutions of **CD28** and **CTLA-4** on the T cell response, **antibody** Fab fragments specific for these molecules were added to SEB stimulated cultures. Addition of **CD28** FAbs inhibited the SEB dependent proliferation. The magnitude of the **CD28** FAb blockade is similar to that observed using anti-**B71**/2 antibodies, implicating **CD28/B7** interactions in providing some costimulation for proliferation in the control cultures. However, there was a...

... This further emphasizes that B7 molecules on APC create an interplay of amplifying signals through **CD28** and attenuating signals through CTLA-4.

CD28 and CTLA-4 Signals Have Opposing Effects on In vivo Expansion of V beta 8 sup + T cells. The effects of anti-**CD28** and anti-**CTLA-4 antibody** treatment on the T cell response to SEB was examined. T cell expansion to superantigens convenient timepoint to initially analyze the affects of anti-**CD28** and anti-CTLA-4 upon the response. Animals were injected with PBS or SEB and...inhibitory effects of intact antibodies was similar to that observed using FAbs, implying that anti-**CD28** antibodies and FAb fragments in vivo both interfere with **B7/CD28** signals. This may be the result of inefficient signaling by bivalent antibody and competition with native ligand by both **antibody** and FAb fragments.

To compare the effects of **CD28** versus **CTLA-4**, anti-**CTLA-4 antibodies** were co-injected with SEB. In contrast to what was observed with anti-**CD28** treatment, administration of anti-CTLA-4 resulted in a dose-dependent increase in accumulation of...

... FAb fragment produced the same result suggest that under these conditions both forms of the **antibody** were blocking **CTLA-4/B7** interactions. Further, the observation that ...inhibitory signal.

Kinetic Analysis ofSEB Responsive Populations. A kinetic analysis was performed to address whether **CD28** and **CTLA-4** affect the magnitude of the response or its timing. An **antibody** dose of 200 mu g/injection was utilized, as this dose was in the range required for saturation of **CD28**, as determined by flow cytometry. The response to SEB and control antibodies was as expected... treated animals had approximately twice as many V beta 8+ T cells relative to control **antibody** treated animals. Finally, to address whether **CTLA-4** or **CD28** present a dominant signal, both **antibodies** were added simultaneously. Throughout the time course, this treatment produced results identical to those obtained with animals treated with anti-**CD28** alone.

B7/CD28 /CTLA-4 Interactions Are Important for Regulating the SEB Response In Vitro. The data presented...

... important for promoting proliferation since blocking with either anti-B7-1/2 antibodies or anti-**CD28** FAb fragments drastically reduced SEB-induced proliferation. In contrast, engagement of **CD28** by intact anti-**CD28** antibodies increases proliferation above the threshold

provided by APC. This increase is probably due to microaggregation or FcR-mediated aggregation of anti-**CD28** **antibodies** leading to efficient crosslinking of **CD28**.

In contrast to **CD28**, **CTLA-4** interactions with **B7** molecules dampens the T cell response to SEB. The observation that anti-CTLA-4 FAb fragments enhance proliferation indicates that CTLA-4/**B7** interactions inhibit proliferative response of T cells to SEB. Further, anti-**B7-1** /2 antibodies augment proliferation in the presence of optimal stimulation with CD28 antibodies, providing additional ...

... presence of a fixed level of TCR signal. There appears to be a requirement for **CD28** signals for optimum responses to SEB; blocking with anti-**CD28** FAb fragments or intact anti-**CD28** **antibodies** effectively diminishes the proliferative expansion. The observation that **CTLA-4** blockade similarly allows increased expansion of responsive cells further supports a similarity in costimulation requirements...

...superantigen and peptide antigen responses in vivo. Further, the kinetic analysis implies that competition between **CD28** and CTLA-4 for **B7**-molecules determines a very early parameter of the T cell ...3 H-thymidine for the final 12 hours prior to harvesting. The inhibitory action of **CTLA-4** appears specific to anti-**CTLA-4** **antibodies** as other T cell binding **antibodies** including anti-L selectin (Mel-14), anti-Thyl.2 and irrelevant antibodies show either no effect or augmentatory effects when co-immobilized with anti-CD3 and anti-**CD28**.

Analysis of Cell Viability: T cells were cultured identically as for proliferation assays. Cell viability... CTLA-4 Engagement Inhibits Proliferation and IL-2 Production. It was previously shown that soluble **antibodies** to **CTLA-4** or **B7** increased thymidine incorporation and IL-2 production by T cells activated by immobilized anti-CD3 and anti-**CD28** in standard three day assays. These results indicated that blockade of CTLA-4/**B7** interactions between the T cells themselves augmented responses by removing inhibitory signals. Since the cultures... The kinetics of the inhibition of proliferation and IL-2 production were examined by crosslinking **CTLA-4** together with CD3 and **CD28** using **antibody** coated microspheres. ...Significant incorporation was detectable by 26 hours in cultures stimulated by anti-CD3 and anti-**CD28** . There was essentially no incorporation detectable at 26 hours when CTLA-4 was also engaged...

2/K/2 (Item 2 from file: 654)

ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
...and an env gp120 carboxy-terminal domain.

FIG. 28 is a FACS analysis showing that **CTLA4**-ova binds immobilized **B7**. The **antibody** in the ELISA assay recognizes and binds the ovalbumin portion of soluble CTLA4-ova.

FIG. 29 is a FACS analysis showing that **CTLA4**-E7 binds immobilized **B7**. The **antibody** in the FACS assay recognizes and binds the E7 portion of soluble CTLA4-E7.

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immobilized **B7**. The **antibody** in the FACS assay recognizes and binds the v3 loop of env gp120 portion of soluble CTLA4-env gp120.

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The human CTLA receptor protein of the...cell interactions with other cells by inhibiting the interaction of CTLA4-positive T cells with **B7** positive cells by reacting the T cells with ligands for the **CTLA4** receptor. The ligands include **B7Ig** fusion protein, a monoclonal **antibody** reactive with **CTLA4** receptor, and **antibody** fragments.

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A monoclonal **antibody** reactive with soluble **CTLA4** fusion protein and a monoclonal **antibody** reactive with soluble **CD28**/**CTLA4** fusion protein are described for use in regulating cellular interactions.

A novel Chinese Hamster Ovary...
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What is claimed is:

1. A monoclonal **antibody** which recognizes and binds the extracellular domain of **CTLA4** , wherein said binding prevents the binding of **CTLA4** to the **B7** antigen.

2. The monoclonal **antibody** of claim 1, wherein the extracellular domain of **CTLA4** is within a CTLA4 fusion protein comprising an amino acid sequence comprising a fragment of the extracellular domain of **CTLA4** which blocks T-cell proliferation.

3. The monoclonal **antibody** of claim 1, wherein the extracellular domain of **CTLA4** is within a CTLA4 fusion protein comprising an amino acid sequence beginning with alanine at...

2/K/3 (Item 3 from file: 654)

ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
...carboxy-terminal domain (SEQ ID NO:18).

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In the ELISA assays, **B7-1** (2.5 mu g/ml) was immobilized to 96 well microtiter plates, samples were blocked...
What is claimed is:

1. A method for regulating CTLA4 positive T cell interactions with **B7** positive B cells comprising contacting **CTLA4**-positive T

cells with a monoclonal **antibody** , Fab or F(ab') sub 2 fragments reactive with **CTLA4** thereby inhibiting interaction of CTLA4-positive T cells with **B7** positive B cells and thus regulating CTLA4-positive T cell interactions with **B7** positive B cells.

2/K/4 (Item 4 from file: 654)
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...a graphic representation of the response of CD4+T cells to costimulation provided by either **B7** (**B7-1**) transfected CHO cells (panel a) or syngeneic activated B lymphocytes (panel b) cultured in media, anti-CD3 alone, or anti-CD3 in the presence of the following monoclonal **antibodies** or recombinant proteins: alpha **B7** (**B7-1**); **CTLA4**-Ig; Fab alpha **CD28** ; or control Ig fusion protein (isotype control for CTLA4Ig); or alpha B5 (the isotype control for anti-**B7**). sup 3 H-Thymidine incorporation was assessed for the last 15 hours of a 72... activated by MHC class II crosslinking. Following activation, cells were harvested and binding of anti-**B7** (CTLA4Ig and mAbs BB-1 and 133), anti-**B7**-3 (CTLA4 and mAb BB-1) and **B7**-2 (CTLA4-Ig) were examined. Results are representative of 25 experiments for **B7** and BB1 monoclonal **antibody** binding and five experiments for **CTLA4**-Ig binding.

FIGS. 7A and 7B are graphic representations of the response of CD4+T...

... in media, anti-CD3 alone, or anti-CD3 in the presence of the following monoclonal **antibodies** or recombinant protein: alpha **B7**(**B7** -**1**); (**CTLA4**-Ig; Fab alpha **CD28**; and alpha B5. sup 3 H-Thymidine incorporation was assessed for the last 15 hours...
... In one embodiment, molecules that can be used to block the interaction of the human **B7**-2 antigen to its natural ligands (e.g., **CTLA4** and **CD28**) include soluble **B7**-2 , **antibodies** that block the binding of **B7** -2 to its ligands and fail to deliver a co-stimulatory signal (so called "blocking antibodies") and **B7**-2 -Ig fusion proteins, which can be produced in accordance with the teaching of the...
... hours following stimulation with either anti-immunoglobulin or anti-MHC class II monoclonal antibody. The **B7**-2 antigen induces detectable IL-2 secretion and T cell proliferation. At about 48 to 72 hours post activation, B cells express both **B7-1** and a third **CTLA4** counter-receptor identified by a monoclonal **antibody** BB-1 (Yokochi, T., et al. (1982) J. Immunol. 128, 823-827), termed **B7**-3. The **B7**-3 antigen is also expressed on **B7** negative activated B cells and can costimulate T cell proliferation without detectable IL-2 production ...crosslinking for 6, 12, 24, 48, 72 and 96 hours were stained with either anti-**B7** (133), BB-1 monoclonal **antibodies**, control IgM **antibody**, **CTLA4**-Ig or control-Ig. Cell suspensions were stained by two step indirect membrane staining with...

... ml of primary monoclonal antibody followed by the appropriate secondary reagents. Specifically, immunoreactivity with anti-**B7** (133) and BB-1 monoclonal antibodies was studied by indirect staining using goat anti-mouse... activated CD4+ T cells proliferated and secreted high levels of IL-2 in response to **B7-1** costimulation provided by CHO-**B7** (panel a). Both proliferation and IL-2 secretion were totally inhibited by blocking the **B7-1** molecule on CHO cells with either anti-**B7-1** monoclonal **antibody** or by a fusion protein for its high affinity receptor, **CTLA4** . Similarly, proliferation and IL-2 secretion were abrogated by blocking **B7-1** signalling via **CD28** with Fab anti-**CD28** monoclonal antibody. Control monoclonal antibody or control fusion protein had no effect. Nearly identical costimulation... 1 monoclonal antibody could completely abrogate both proliferation and IL-2 secretion delivered by CHO-**B7**, anti-**B7-1** monoclonal antibody consistently inhibited proliferation induced by activated B cells by only 50% whereas IL...

... secretion was totally inhibited. In contrast to the partial blockage of proliferation induced by anti-**B7-1** monoclonal **antibody**, both **CTLA4**-Ig and Fab anti-**CD28** monoclonal **antibody** completely blocked proliferation and IL-2 secretion. Identical results were obtained when the responding T cell population was **CD28**+ T cells and when PMA was used to deliver the first submitogenic signal rather than... 3a). FIG. 3b displays the costimulatory potential of B7- activated human splenic B cells. Irradiated **B7**- activated (72 hr) B cells could also deliver a significant costimulatory signal to submitogenically activated...

...B7- activated splenic B cells confirmed the above functional results. As seen in FIG. 4, **B7**+ activated splenic B cells stained with anti-**B7-1** (133) monoclonal **antibody**, BB-1 monoclonal **antibody**, and bound **CTLA4**-1g. In contrast, **B7**- activated splenic B cells did not stain with anti-**B7-1** (133) monoclonal **antibody** but did stain with BB-1 monoclonal **antibody** and **CTLA4** -Ig. These phenotypic and functional results demonstrate that both **B7**+ and **B7** - activated (72 hours) human B lymphocytes express CTLA4 binding counter- ...2 secretion; and 2) are identified by the BB-1 monoclonal antibody but not anti-**B7-1** monoclonal **antibody**.

Example 3: Three Distinct **CTLA4/CD28** Ligands Are Expressed Following Human B Cell Activation

To determine the sequential expression of CTLA4...sup 3 H-Thymidine incorporation). Neither proliferation nor IL-2 accumulation was inhibited by anti-**B7-1** (133) or BB-1. In contrast with **CTLA4**-Ig and Fab anti-**CD28** monoclonal **antibody** totally abrogated proliferation and IL-2 accumulation. B cells activated for ... costimulation which resulted in nearly maximal proliferation and IL-2 secretion (FIG. 7b). Here, anti-**B7-1** (133) monoclonal antibody, inhibited proliferation approximately 50% but totally blocked IL-2 accumulation. BB-1 monoclonal **antibody** totally inhibited both proliferation and IL-2 secretion. As above, **CTLA4**-Ig and Fab anti-**CD28** also totally blocked proliferation and IL-2 production. Finally, 72 hour activated B cells induced...133) monoclonal antibodies. They also demonstrated that these BB-1 positive cells did not express **B7** mRNA yet bound **CD28** transfected COS cells providing further support for the existence of a distinct protein which binds monoclonal antibody BB-1.

Our present findings confirm that there is an additional **CTLA4** counter-receptor identified by the BB-1 monoclonal **antibody**, **B7-3**, and that this protein appears to be functionally distinct from **B7-1** (133). Although the expression of **B7-1** and **B7-3** following B cell activation appears to be concordant on **B7** positive B cells, these studies demonstrate that the B7-3 molecule is also expressed on...and isolated mononuclear cells produce detectable levels of IL-2 in vitro. Therefore, an alternative **CD28** costimulatory counter-receptor or an alternative IL-2 producing pathway must be ...the most effective reagents to induce antigen specific anergy in murine and human systems are **CTLA4**-Ig and Fab anti-**CD28**, whereas anti-**B7** monoclonal **antibodies** have been much less effective (Harding, F. A., et al. (1992) Nature. 356, 607-609...

2/K/5 (Item 5 from file: 654)

...have found that the inhibition of T-dependent immune responses resulting from blockade of either **CD28** or CD40 signals is potent, but incomplete. The data herein demonstrate that simultaneous blockade of...

... chronic rejection of transplanted tissue in vivo. Independent blockade of these pathways using a soluble **CTLA4** molecule or **antibodies**

which recognize and bind gp39 failed to even minimally prolong survival of primary skin transplanted tissue.

The invention herein involves the discovery that simultaneous blockade of **CD28** and CD40 signals promoted long-term survival of fully allogeneic as well as xenogeneic skin...
...its ligand as described above. This example provides the additional step of preventing the endogenous **B7** antigen from binding its endogenous ligand which comprises contacting a CTLA4-positive cell with a...

... ligand which recognizes and binds the CTLA4 antigen. Examples of such soluble ligands include soluble **B7** molecules and **antibodies** directed against **CTLA4**.

The binding of the CD40-positive cell to the soluble ligand blocks the reaction of CD40 antigen with endogenous gp39. Additionally, the binding of the CTLA4- or **CD28** -positive cell to the soluble ligand blocks the reaction of the CTLA4 antigen with endogenous...CD40.

In one example, the second binding domain is a ligand which recognizes and binds **CTLA4**. Examples include **B7** and monoclonal **antibodies** directed against **CTLA4** . In another example, the second binding domain is a ligand which recognizes and binds the **CD28** antigen. Examples include **B7** and monoclonal antibodies directed against **CD28** . In another example, the second binding domain is a ligand which recognizes and binds the **B7** antigen. Examples include **CTLA4**, **CD28** and monoclonal **antibodies** directed against **B7**.

Soluble ligands may be administered during transplant, before transplant, or after transplant. Soluble ligands may... 20 days after receiving a Sprague-Dawley rat heart graft (FIG. 9B). Treatment with either **CTLA4** -Ig or MR1 alone decreased the IgG **antibody** response, whereas the simultaneous combination **CD28** /CD40 blockade essentially eliminated the evoked antibody response to rat xenoantigens. Thus, inhibition of both ...

... and antibody production could be functionally important in xenograft survival after simultaneous blockade of the **B7/CD28** and CD40/gp39 pathways.

Discussion

Combined blockade of the CD28 and CD40 pathways markedly inhibits...
... claim 1 or 3, wherein said second soluble ligand is a monoclonal antibody directed against **CD28**.

14. The method of claim 1 or 3, wherein said second soluble ligand is a monoclonal **antibody** directed against **CTLA4**.

15. The method of claim 1, 2, or 4, wherein the second soluble ligand is ...

... The method of claim 1, 2, or 4, wherein the second soluble ligand is soluble **CD28**.

17. The method of claim 15, wherein the soluble CTLA4 is a recombinant binding molecule...

2/K/6 (Item 6 from file: 654)

ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments

and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
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2/K/7 (Item 7 from file: 654)

ABSTRACT

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... further includes a method for treating immune system diseases mediated by T cell interactions with **B7** positive cells by administering a ligand reactive with **B7** antigen to regulate T cell interactions with **B7** positive cells. The ligand is the CTLA4Ig fusion protein, or the **CD28**/CLA4Ig fusion protein hybrid, or a monoclonal **antibody** reactive with **B7** antigen.

A monoclonal **antibody** reactive with soluble **CTLA4** fusion protein and a monoclonal **antibody** reactive with soluble **CD28**/**CTLA4** fusion protein are described for use in regulating cellular interactions.

A novel Chinese Hamster Ovary...
... a more potent inhibitor in vitro of lymphocyte responses than either anti-BB1, or anti-**CD28** mAbs. CTLA4Ig does not have direct stimulatory effects on T cell proliferation to counteract its...

... Therefore, the CTLA4 fusion proteins may perform as a better inhibitor in vivo than anti-**CD28** monoclonal **antibodies** . The immunosuppressive effects of **CTLA4** fusion proteins (e.g., CTLA4Ig) in vitro suggests its use in therapy or treatment of...of the invention, other reagents, including derivatives reactive with the CTLA4Ig fusion protein or the **CTLA4** receptor are used to regulate T cell interactions. For

example, **antibodies**, and/or **antibody** fragments reactive with the **CTLA4** receptor can be screened to identify those capable of inhibiting the binding of the soluble **CTLA4** fusion protein to the **B7** antigen. The **antibodies** or **antibody** fragments such as Fab or F(ab') sub 2 fragments, may then be used to react with the T cells, for example, to inhibit T cell proliferation.

Anti-**B7** monoclonal antibodies prepared as described above can be used to bind to **B7** antigen to inhibit interactions of **CD28** -positive or **CTLA4**-positive T cells with **B7** positive cells. Anti-**CTLA4** monoclonal **antibodies** can be used to bind to **CTLA4** receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

In another...

... be used to identify additional compounds capable of regulating the interaction between CTLA4 and the **B7** antigen. Such compounds may include small naturally occurring molecules that can be used to react...we have identified regions in CTLA4Ig which are required for its high avidity binding to **B7-1**. The following is a description of how to make soluble CTLA4/**CD28** hybrid fusion proteins which bind **B7**.

MATERIALS AND METHODS

Monoclonal **antibodies** (mAbs)

Murine mAb's specific for **CTLA4** were prepared and characterized as previously described (Linsley et al. J. Ex. Med., (1992) 176...122 (Table I and FIG. 17).

HS4-B and HS4-A displayed similar binding to **B7-1**. Unlike HS4-A, however, the inclusion of the CTLA4 CDR1-like loop into HS4-B... ... immediately adjacent to the CTLA4Ig MYPPPY motif were important determinants in high avidity binding.

Monoclonal **antibody** binding to **CTLA4**/CD28Ig hybrid fusion proteins

The structural integrity of each hybrid fusion protein was examined by assessing their ability to bind mab's specific for CTLA4 or **CD28** in an enzyme immunoassay. The CTLA4 specific mab's 7F8, 11D4 and 10A8 block ligandBinding of **CTLA4** and CD26 monoclonal **antibodies** to CTLA4Ig and CD28Ig mutant fusion proteins and to **CTLA4**/CD28Ig hybrid fusion proteins.

anti-CTLA4 mAbs | anti-**CD28** mAb

7F8 | 11D4 | 10A8 | 9.3...were used (M. Kahn et al. J. Immunol. (1991) 146(9):3235-41).

The soluble **CTLA4** fusion protein/**antibody** complex was in turn visualized with a FITC-labelled second antibody. Binding of all the...

...proteins to the B cells was competitively inhibited by soluble CTLA4Ig.
In the ELISA assays, **B7-1** (2.5 mu g/ml) was immobilized to 96 well microtiter plates, samples were blocked...

2/K/8 (Item 8 from file: 654)
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... a pharmaceutical composition can contain other biologically active molecules, for example, lymphokines, cytokines, other monoclonal **antibodies** or fusion proteins (i.e., **CD28**-Ig, **CTLA4**-Ig).

The monoclonal **antibodies** , recombinant binding proteins and

pharmaceutical compositions thereof of this invention are particularly useful for oral...

2/K/9 (Item 9 from file: 654)
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...can be a selected immune modulator in this method.

Alternatively, an agent which blocks the **CD28** and/or CTLA4 ligands present on T helper cells interferes with the normal binding of those ligands with the antigen **B7** on the B cell. Thus, a soluble form of **B7** or an **antibody** to **CD28** or **CTLA4**, e.g., **CTLA4**-Ig [available from Bristol-Myers Squibb Co; see, e.g., European patent application 606,217...

2/K/10 (Item 10 from file: 654)
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... in an environment wherein other substances in the same environment are not complexed to the **B7** antigen. The complex is formed in a manner that blocks the normal signal transduction pathway of **B7** through the **CD28** or CTLA4 antigen. Molecules which bind to the **B7** antigen include **CD28**, **CTLA4**, CTLA4Ig and anti-**B7** **antibodies**
.

As used herein, the term "**antibody** " refers to polyclonal antibodies, monoclonal antibodies, humanized antibodies, single-chain antibodies, and fragments thereof such...GVHD, or rheumatoid arthritis. The two components are: (1) a molecule that binds to the **B7** antigen such as MAb **B7**-24; and (2) an immunosuppressive agent.

Molecules that bind to the **B7** antigen include **CD28**, **CTLA4**, CTLA4Ig and anti-**B7** **antibodies**.

1. **Antibody** Preparation

Monoclonal **antibody** **B7**-24 is prepared as described in Example 1 herein. Other monoclonal antibodies of the invention may be prepared similarly, or as follows. First, polyclonal antibodies are raised against the **B7** antigen. Second, monoclonal antibodies specific for **B7** are selected.

a. Polyclonal Sera

Polyclonal sera may be prepared by conventional methods. In general... ...such treatment a therapeutically effective amount of (a) a molecule that specifically binds to the **B7** antigen, said molecule upon binding to the **B7** antigen, blocking the normal signal transduction pathway of **B7** through the **CD28** or **CTLA4** pathways, said molecule being an **antibody** or an antigen binding fragment thereof; and (b) an immunosuppressive agent in a pharmaceutically acceptable excipient.

2. The method of claim 1, wherein the molecule that binds to the **B7** antigen is an anti-**B7** antibody.

3. The method of claim 2, wherein the anti-B7 antibody is a monoclonal... such treatment a therapeutically effective amount of (a) a molecule that specifically binds to the **B7** antigen, said molecule upon binding to the **B7** antigen, blocking the normal signal transduction pathway of **B7** through the **CD28** or **CTLA4** pathways, said molecule being an **antibody** or an antigen binding fragment thereof; and (b) an immunosuppressive agent in a pharmaceutically acceptable excipient.

9. The method of claim 8, wherein the molecule that binds to the **B7**

antigen is an anti-**B7** antibody.

10. The method of claim 9, wherein the anti-B7 antibody is a monoclonal ...such treatment a therapeutically effective amount of (a) a molecule that specifically binds to the **B7** antigen, said molecule upon binding to the **B7** antigen, blocking the normal signal transduction pathway of **B7** through the **CD28** or **CTLA4** pathways, said molecule being an **antibody** or an antigen binding fragment thereof; and (b) an immunosuppressive agent in a pharmaceutically acceptable excipient.

16. The method of claim 15, wherein the molecule that binds to the **B7** antigen is an anti-**B7** antibody.

17. The method of claim 16, wherein the anti-B7 antibody is a monoclonal ...

2/K/11 (Item 11 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... hours following stimulation with either anti-immunoglobulin or anti-MHC class II monoclonal antibody. The **B7**-2 antigen induces detectable IL-2 secretion and T cell proliferation. At about 48 to 72 hours post activation, human B cells express both **B7** and a third **CTLA4** counter-receptor, **B7**-3, identified by a monoclonal **antibody** BB-1, which also binds **B7** (Yokochi, T., et al. (1982) J. Immunol. 128, 823-827). The **B7**-3 antigen is also expressed on **B7** negative activated B cells and can costimulate T cell proliferation without detectable IL-2 production...

2/K/12 (Item 12 from file: 654)
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... in vivo growth of the tumor cell line V51Blim10 in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28** . FIG. 1B is a graph illustrating the average tumor size in mice injected with 2X10...

...injected with V51Blim10 cells.

FIG. 2 is a graph showing the in vivo growth of **B7**-51BL10 tumors in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28**.

FIG. 3 shows the rejection of wild-type colon carcinoma cells by mice previously treated with V51BLim10 cells and anti-**CTLA-4 antibody**.

FIG. 4 shows the growth of established tumors after treatment with anti-**CTLA-4 antibody**.

FIG. 5 shows the growth of the murine firbosarcoma SA1N in the absence or presence...
...but not CD4lg in ELISAs; 2) the ability to block CTLA-41 lg binding to **B7** transfectants; 3) the ability to stain activated T cells but not freshly isolated T cells; and 4) the ability to stain a **CTLA-4** transfectant but not control transfectants.

The ability of **antibody** 9H10 to block CTLA41 lg binding to **B7** transfectants was demonstrated as follows. Approximately 10 mu l of mAb 9H10 was incubated at... 10,000 live gated events were collected for analysis. The results showed that the 9H10 **antibody** blocked **CTLA-4** binding to **B7**-EL-4 cells.

The ability of the 9H10 antibody to stain activated T cells but...tumors

were measured with calipers. All of the mice left untreated, or treated with anti-**CD28** antibody, developed progressively growing tumors and required euthanasia by 35 days after inoculation. In contrast, all mice treated with anti-**CTLA-4** **antibody** completely rejected their tumors after a short period of limited growth. As shown in FIG...with calipers. The data from this experiment is shown in FIG. 2. Treatment with anti-**CTLA-4** **antibodies** inhibited **B7**-51BLim10 tumor growth as compared to the anti-**CD28** and control groups all mice in the untreated and anti-**CD28** treated groups developed small tumors that grew progressively for five to ten days and then...

2/K/13 (Item 13 from file: 654)

ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
...and an env gp120 carboxy-terminal domain.

FIG. 28 is a FACS analysis showing that **CTLA4**-ova binds immobilized **B7**. The **antibody** in the ELISA assay recognizes and binds the ovalbumin portion of soluble CTLA4-ova.

FIG. 29 is a FACS analysis showing that **CTLA4**-E7 binds immobilized **B7**. The **antibody** in the FACS assay recognizes and binds the E7 portion of soluble CTLA4-E7.

FIG. 30 is a FACS analysis showing that **CTLA4**-env gp120 binds immobilized **B7**. The **antibody** in the FACS assay recognizes and binds ...gp120 portion of soluble CTLA4-env gp120.

FIG. 31 is a FACS analysis showing that **CTLA4**-p97 binds immobilized **B7**. The **antibody** in the FACS assay recognizes and binds the p97 portion of soluble CTLA4-p97.

FIG. 32 is a line graph showing that soluble CTLA4-ova (closed circle) binds immobilized **B7** in an ELISA assay. The antibody in the ELISA assay recognizes and binds the ovalbumin...a more potent inhibitor in vitro of lymphocyte responses than either anti-BB1, or anti-**CD28** mAbs. CTLA4Ig does not have direct stimulatory effects on T cell proliferation to counteract its...

... Therefore, the CTLA4 fusion proteins may perform as a better inhibitor in vivo than anti-**CD28** monoclonal **antibodies** . The immunosuppressive effects of **CTLA4** fusion proteins (e.g., CTLA4Ig) in vitro suggests its use in therapy for treatment of...of the invention, other reagents, including derivatives reactive with the CTLA4Ig fusion protein or the **CTLA4** receptor are used to regulate T cell interactions. For example, **antibodies**, and/or **antibody** fragments reactive with the **CTLA4** receptor can be screened to identify those capable of inhibiting the binding of the soluble **CTLA4** fusion protein to the **B7** antigen. The **antibodies** or **antibody** fragments such as Fab or F(ab') sub 2 fragments, may then be used to react with the T cells, for example, to inhibit T cell proliferation.

Anti-**B7** monoclonal antibodies prepared as described above can be used to bind to **B7** antigen to inhibit interactions of **CD28** -positive or **CTLA4**-positive T cells with **B7** positive cells. Anti-**CTLA4** monoclonal **antibodies** can be used to bind to **CTLA4** receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

In another...

... be used to identify additional compounds capable of regulating the interaction between CTLA4 and the **B7** antigen. Such compounds may include small naturally occurring molecules that can be used to react...
... product. Embodiments of the invention include CTLA4Ig fusion protein, and hybrid (chimeric) fusion proteins including **CD28**/CTLA4Ig fusion proteins (which is also referred to herein as the CTLA4/CD28Ig fusion protein). Also provided are methods for using the CTLA4 fusion protein, **B7Ig** fusion protein, hybrid fusion proteins, and fragments and/or derivatives thereof, such as monoclonal **antibodies** reactive with **CTLA4** and the **B7** antigen, to regulate cellular interactions and immune responses.

The human CTLA receptor protein of the...cell interactions with other cells by inhibiting the interaction of CTLA4-positive T cells with **B7** positive cells by reacting the T cells with ligands for the **CTLA4** receptor. The ligands include **B7Ig** fusion protein, a monoclonal **antibody** reactive with **CTLA4** receptor, and **antibody** fragments.

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A monoclonal **antibody** reactive with soluble **CTLA4** fusion protein and a monoclonal **antibody** reactive with soluble **CD28**/**CTLA4** fusion protein are described for use in regulating cellular interactions.

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anti-CTLA4 mAbs
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The soluble **CTLA4** fusion protein/**antibody** complex was in turn visualized with a FITC-labelled second antibody. Binding of all the...

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In the ELISA assays, **B7-1** (2.5 mu g/ml) was immobilized to 96 well microtiter plates, samples were blocked...

2/K/14 (Item 14 from file: 654)

ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
...carboxy-terminal domain (SEQ ID NO:18).

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2/K/15 (Item 15 from file: 654)

... in vivo growth of the tumor cell line V51Blim10 in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28** . FIG. 1B is a graph illustrating the average tumor size in mice injected with 2X10...

...injected with V51Blim10 cells.

FIG. 2 is a graph showing the in vivo growth of **B7**-51BLim10 tumors in the presence or absence of **antibodies** directed against **CTLA-4** or **CD28**.

FIG. 3 shows the rejection of wild-type colon carcinoma cells by mice previously treated with V51BLim10 cells and anti-**CTLA-4** **antibody**.

FIG. 4 shows the growth of established tumors after treatment with anti-**CTLA-4** **antibody**.

FIG. 5 shows the growth of the murine fibrosarcoma SA1N in the absence or presence of anti-**CTLA-4** **antibodies**.
FIGS. 6A to 6F illustrate the adjuvant effect of anti-**CTLA-4** **antibodies** in the response of T cells to peptide antigens.

FIGS. 7A to 7F illustrate the of CTLA-4/**B7** blockade on the proliferation of purified CD4+ T cells. In FIG. 8B, detection of IL...
...4lg but not CD4lg in ELISAs; 2) the ability to block CTLA-4lg binding to **B7** transfectants; 3) the ability to stain activated T cells but not freshly isolated T cells; and 4) the ability to stain a **CTLA-4** transfectant but not control transfectants.

The ability of **antibody** 9H10 to block CTLA4lg binding to **B7** transfectants was demonstrated as follows. Approximately 10 mu l of mAb 9H10 was incubated at... 10,000 live gated events were collected for analysis. The results showed that the 9H10 **antibody** blocked **CTLA-4** binding to **B7**-EL-4 cells.

The ability of the 9H10 antibody to stain activated T cells but...tumors were measured with calipers. All of the mice left untreated, or treated with anti-**CD28** antibody, developed progressively growing tumors and required euthanasia by 35 days after inoculation. In contrast, all mice treated with anti-**CTLA-4** **antibody** completely rejected their tumors after a short period of limited growth. As shown in FIG...with calipers. The data from this experiment is shown in FIG. 2. Treatment with

anti-**CTLA-4** **antibodies** inhibited **B7**-51BLim10 tumor growth as compared to the anti-**CD28** and control groups. All mice in the untreated and anti-**CD28** treated groups developed small tumors that grew progressively for five to ten days and then...in vitro and that signals through CTLA-4 inhibit the response. In vivo, blockade of **CD28** by FAb fragments or intact antibodies have the opposite effects upon V beta 8+ expansion to a similar blockade with anti-**CTLA-4** FAb fragments or intact **antibodies**. Analysis of the kinetics of the expansion imply that signals through **CD28** promote T cell expansion, whereas an opposing signal through CTLA-4 functions during T cell...96 well round bottom plates. SEB was added at the indicated concentrations. Where indicated, anti-**CD28** was added at a 1:1000 dilution of ascites, anti-**B7-1** was added at 5 mu g/ml and anti-**B7**-2 was added at 20 mu g/ml, and equal quantities of non-specific control **antibody** 560.31 were added. For FAb experiments, anti-**CD28**, anti-**CTLA-4** or control FAb fragments were added at 100 mu g/ml. Cultures were incubated for... B7-1/B7-2 induced a slight but reproducible increase in proliferation compared to anti-**CD28** by itself, suggesting that another **B7** ligand besides **CD28** (i.e. CTLA-4) might be important in downregulating the response of T cells to SEB.

To address the relative contibutions of **CD28** and **CTLA-4** on the T cell response, **antibody** Fab fragments specific for these molecules were added to SEB stimulated cultures. Addition of **CD28** FAbs inhibited the SEB dependent proliferation. The magnitude of the **CD28** FAb blockade is similar to that observed using anti-**B71**/2 antibodies, implicating **CD28**/**B7** interactions in providing some costimulation for proliferation in the control cultures. However, there was a...

... This further emphasizes that B7 molecules on APC create an interplay of amplifying signals through **CD28** and attenuating signals through CTLA-4.

CD28 and CTLA-4 Signals Have Opposing Effects on In vivo Expansion of V sub beta 8 sup + T cells. The effects of anti-**CD28** and anti-**CTLA-4** **antibody** treatment on the T cell response to SEB was examined. T cell expansion to superantigens...inhibitory effects of intact antibodies was similar to that observed using FAbs, implying that anti-**CD28** antibodies and FAb fragments in vivo both interfere with **B7**/**CD28** signals. This may be the result of inefficient signaling by bivalent antibody and competition with native ligand by both **antibody** and FAb fragments.

To compare the effects of **CD28** versus **CTLA-4**, anti-**CTLA-4** **antibodies** were co-injected with SEB. In contrast to what was observed with anti-**CD28** treatment, administration of anti-CTLA-4 resulted in a dose-dependent increase in accumulation of...FAb fragment produced the same result suggest that under these conditions both forms of the **antibody** were blocking **CTLA-4**/**B7** interactions. Further, the observation that an increase in V beta 8+ cells was observed under...

...signal.

Kinetic Analysis of SEB Responsive Populations. A kinetic analysis was performed to address whether **CD28** and **CTLA-4** affect the magnitude of the response or its timing. An **antibody** dose of 200 mu g/injection was utilized, as this dose was in the range required for saturation of **CD28**, as determined by flow cytometry. The response to SEB and control antibodies was as expected... treated animals had approximately twice as many V beta 8+ T cells relative to control **antibody** treated animals. Finally, to address whether **CTLA-4** or **CD28** present a dominant signal, both **antibodies** were added simultaneously. Throughout the time course, this treatment produced results identical to those obtained with animals treated with anti-

CD28 alone.

B7/CD28 /CTLA-4 Interactions Are Important for Regulating the SEB Response In Vitro. The data presented...
... important for promoting proliferation since blocking with either anti-B7-1/2 antibodies or anti-CD28 FAb fragments drastically reduced SEB-induced proliferation. In contrast, engagement of CD28 by intact anti-CD28 antibodies increases proliferation above the threshold provided by APC. This increase is probably due to microaggregation or FcR-mediated aggregation of anti-CD28 antibodies leading to efficient crosslinking of CD28.

In contrast to CD28, CTLA-4 interactions with B7 molecules dampens the T cell response to SEB. The observation that anti-CTLA-4 FAb fragments enhance proliferation indicates that CTLA4/B7 interactions inhibit proliferative response of T cells to SEB. Further, anti-B7-1 /2 antibodies augment proliferation in the presence of optimal stimulation with CD28 antibodies, providing additional ...

... presence of a fixed level of TCR signal. There appears to be a requirement for CD28 signals for optimum responses to SEB; blocking with anti-CD28 FAb fragments or intact anti-CD28 antibodies effectively diminishes the proliferative expansion. The observation that CTLA-4 blockade ...superantigen and peptide antigen responses in vivo. Further, the kinetic analysis implies that competition between CD28 and CTLA-4 for B7-molecules determines a very early parameter of the T cell response; in this experiment a...3 H-thymidine for the final 12 hours prior to harvesting. The inhibitory action of CTLA-4 appears specific to anti-CTLA-4 antibodies as other T cell binding antibodies including anti-L selectin (Mel-14), anti- ...show either no effect or augmentatory effects when co-immobilized with anti-CD3 and anti-CD28.

Analysis of Cell Viability: T cells were cultured identically as for proliferation assays. Cell viability... CTLA-4 Engagement Inhibits Proliferation and IL-2 Production. It was previously shown that soluble antibodies to CTLA-4 or B7 increased thymidine incorporation and IL-2 production by T cells activated by immobilized anti-CD3 and anti-CD28 in standard three day assays. These results indicated that blockade of CTLA-4/B7 interactions between the T cells themselves augmented responses by removing inhibitory signals. Since the cultures...

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... reactive with the CTLA4 mutant molecule are used to regulate T cell interactions. For example, antibodies, and/or antibody fragments reactive with the CTLA4 receptor may be screened to identify those capable of inhibiting the binding of the CTLA4 mutant molecule to the B7-1 antigen. The antibodies or antibody fragments such as Fab or F(ab') sub 2 fragments, may then be used to...we have identified regions in CTLA4Ig which are required for its high avidity binding to B7-1. The following is a description of how to make soluble CTLA4/CD28 hybrid fusion proteins which bind B7.

Materials and Methods

Monoclonal antibodies (mAbs). Murine Mab's specific for CTLA4 were prepared and characterized as previously described (Linsley et al. J. Ex. Med., (1992) 176:1595-1604). Antibody 9.3 (anti-CD28) has been described previously ((Hansen et al., Immunogenetics 10:247-260 (1980)).

Cell Culture. The... CTLA4Ig MYPPPY (SEQ ID NO 11) motif were important determinants in high avidity binding.

Monoclonal **antibody** binding to **CTLA4**/CD28Ig hybrid fusion proteins. The structural integrity ...protein was examined by assessing their ability to bind mAb's specific for CTLA4 or **CD28** in an enzyme immunoassay. The CTLA4 specific mAb's 7F8, 11D4 and 10A8 block ligand Binding of **CTLA4** and **CD28** monoclonal **antibodies** to CTLA4Ig and CD28Ig mutant fusion proteins and to **CTLA4**/CD28Ig hybrid fusion proteins.

anti-CTLA4 mAbs		anti-**CD28** mAb	
7F8	11D4	10A8	9.3...

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ABSTRACT

... fusion proteins, and for using the soluble fusion proteins, fragments and derivatives thereof, including monoclonal **antibodies** reactive with **B7** and **CTLA4**, to regulate T cell interactions and immune responses mediated by such interactions.
... including CD28Ig/CTLA4Ig fusion proteins. Also provided are methods for using the CTLA4 fusion protein, **B7Ig** fusion protein, hybrid fusion proteins, and fragments and/or derivatives thereof, such as monoclonal **antibodies** reactive with **CTLA4** and the **B7** antigen, to regulate cellular interactions and immune responses.

The human CTLA receptor protein of the...cell interactions with other cells by inhibiting the interaction of CTLA4-positive T cells with **B7** positive cells by reacting the T cells with ligands for the **CTLA4** receptor. The ligands include **B7Ig** fusion protein, a monoclonal **antibody** reactive with **CTLA4** receptor, and **antibody** fragments.

The invention also provides a method for regulating T cell interactions with **B7** positive cells, using a ligand for the **B7** antigen. Such a ligand is the CTLA4Ig fusion protein of the invention, its fragments or ...
... of the invention, other reagents, including derivatives reactive with the CTLA4Ig fusion protein or the **CTLA4** receptor are used to regulate T cell interactions. For example, **antibodies**, and/or **antibody** fragments reactive with the **CTLA4** receptor may be screened to identify those capable of inhibiting the binding of the CTLA4Ig fusion protein to the **B7** antigen. The antibodies or antibody fragments such as Fab or F(ab') sub 2 fragments...

... used to react with the T cells, for example, to inhibit T cell proliferation.

Monoclonal **antibodies** reactive with **CTLA4** receptor, may be produced by hybridomas prepared using known procedures, such as those introduced by... e.g. Rousseaux et al., in Methods Enzymol., 121:663-69, Academic Press (1986)).

Anti-**B7** monoclonal antibodies prepared as described above may be used to bind to **B7** antigen to inhibit interactions of **CD28**-positive or **CTLA4**-positive T cells with B7 positive cells. Anti-**CTLA4** monoclonal **antibodies** may be used to bind to **CTLA4** receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

In another...

... be used to identify additional compounds capable of regulating the

interaction between CTLA4 and the **B7** antigen. Such compounds may include small naturally occurring molecules that can be used to react...

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... IL2 receptor, monoclonal antibodies and receptor fusion proteins which antagonize the CD40/gp39 interaction and **CTLA 4** -Ig in monoclonal **antibodies** which antagonize the **B7/CD28** interaction. Also, in the case of treatment of rheumatoid arthritis, the subject antibody may be...

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... in an environment wherein other substances in the same environment are not complexed to the **B7-1** antigen. The complex is formed in a manner that blocks the normal signal transduction pathway of **B7-1** through the **CD28** or CTLA4 antigen. Molecules which bind to the **B7** antigen include **CD28**, **CTLA4**, CTLA4Ig and anti-**B7 antibodies**.

II. Generating **Antibodies** to Membrane-Associated Antigen Molecules

This section describes a method for generating and selecting antibodies ...of the molecule. In addition, solubilization of proteins often decreases their immunogenicity. Therefore, most monoclonal **antibodies** to cell surface antigens have been obtained after immunization with mice with ... GVHD, or rheumatoid arthritis. The two components are: (1) a molecule that binds to the **B7-1** antigen such as MAb **B7**-24; and (2) an immunosuppressive agent. Molecules that bind to the **B7-1** antigen include **CD28**, **CTLA4**, CTLA4Ig and anti-**B7 antibodies** as described in Section III above.

The anti **B7-1** antibodies of the invention (or other molecules that bind to the **B7**-1 antigen) are given in combination with one or more immunosuppressive agents. Immunosuppressive agents are Monoclonal antibody **B7**-24 binds to a different antigenic epitope on the **B7-1** molecule than the BB-1 monoclonal **antibody** and the **CTLA-4** Ig fusion protein. **B7**-24 does not bind to **B7**-2, whereas CTLA-4 Ig does; **B7**-24 and CTLA-4 Ig do not bind to **B7-1** negative cells, which are positive for staining with BB-1 monoclonal antibody. [Boussiotis et al...same day of the grafting is not reported to result in tolerance. Thus, signaling by **B7**-2 interaction with T cells is needed for tolerance induction and the blocking effect at day 2 is due to blocking **B7-1**. With the **B7**-24 **antibody**, this is not a problem because in contrast to **CTLA-4** Ig, it does not block **B7** -2. With respect to tolerance induction versus suppression combination of anti-**B7-1** with CsA is not obvious signal transduction through the TcR/CD3 complex and anti-**B7** -2 are needed for tolerance induction. This means that the signals by the TcR/CD3...

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...Aug. 18, 1993] can be a selected immune modulator.

Alternatively, an agent which blocks the CD28 and/or CTLA4 ligands present on T helper cells interferes with the normal binding of those ligands with the antigen **B7** on the B cell. Thus, a soluble form of **B7** or an **antibody** to **CD28** or **CTLA4**, e.g., **CTLA4**-Ig [available from Bristol-Myers Squibb Co;

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... receptor. For example, the DNA sequence may encode the variable region or regions of an **antibody** which recognizes and binds the BR96 antigen, CD3, L6, **CD28**, **CTLA4**, or **B7**. Additionally, the DNA sequence may encode variable regions capable of binding to other cell surface...
...13. The expression vector of claim 1, wherein the first antibody binding domain binds to **CD28** and the second antibody binding domain binds to L6.

14. The expression vector of claim 1, wherein the first **antibody** binding domain binds to **CTLA4** and the second **antibody** binding domain binds to L6.

15. The expression vector of claim 1, wherein the first antibody binding domain binds to the **B7** molecule and the second antibody binding domain binds to L6.

16. An expression vector encoding...

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ABSTRACT

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The invention also provides a method for regulating T cell interactions with **B7** positive cells, using a ligand for the **B7** antigen.
... of the invention, other reagents, including derivatives reactive with the CTLA4Ig fusion protein or the **CTLA4** receptor are used to regulate T cell interactions. For example, **antibodies**, and/or **antibody** fragments reactive with the **CTLA4** receptor may be screened to identify those capable of inhibiting the binding of the CTLA4Ig fusion protein to the **B7** antigen. The antibodies or antibody fragments such as Fab or F(ab') sub 2 fragments...

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Anti-**B7** monoclonal antibodies prepared-as described above may be used to bind to **B7** antigen to inhibit interactions of **CD28** -positive or **CTLA4**-positive T cells with **B7** positive cells. Anti-**CTLA4** monoclonal **antibodies** may be used to bind to **CTLA4** receptor to inhibit the interaction of CTLA4-positive T cells with other cells.

In another...

... be used to identify additional compounds capable of regulating the interaction between CTLA4 and the **B7** antigen. Such compounds may include small naturally occurring molecules that can be used to react...